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EFFECTIVENESS OF REPELLENTS AGAINST SEVERAL SPECIES OF ANOPHELES MOSQUITOES[1]

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The military needs for insect repellents during World War II stimulated a number of screening programs to select more effective materials. At the Orlando, Fla., laboratory of the Bureau of Entomology and Plant Quarantine from 1942 to 1945, when the program was active, preliminary tests were made with several thousand materials applied to the skin for protection against *Anopheles quadrimaculatus* Say. Most of these materials were received from the manufacturers, but many were prepared by chemists in the Bureau and in the following universities: Harvard, Maryland, Minnesota, Ohio State, Stanford, Columbia, Illinois, and Wisconsin. In 1945 a number of the more promising materials were tested in the Portland, Oreg., laboratory of the Bureau against *A. freeborni* Aitken and *A. punctipennis* (Say), and various cooperators tested a few of the better materials against other *Anopheles* species in Panama, Trinidad, and Africa. The results of these tests are summarized in this paper.

METHODS

All tests against *Anopheles quadrimaculatus*, *A. freeborni*, and *A. punctipennis* were made in the laboratory. Each repellent was applied to the arm (elbow to wrist) of a test subject, and the arm was exposed to the caged mosquitoes. Exposures were made immediately after treatment and at intervals of 20 to 30 minutes until a bite was received. Mosquitoes were added to the cages daily so that the testing population would be composed of adults of various ages. Enough mosquitoes were maintained in each cage to provide a biting rate of 5 to 20 per half-minute. The evaluation of all materials was based on the time from application to the first bite and recorded as repellent time. Frequently it was necessary to terminate tests before bites were received. Where the repellent times for such tests exceeded the average of completed tests, they were included in the average (in the tables such averages are marked with a plus sign).

Because *Anopheles quadrimaculatus* was found to have extremely erratic biting habits under cage conditions, the tests against this species were replicated many times to insure more accurate results.

[1] This work was conducted under a transfer of funds, recommended by the Committee on Medical Research, from the Office of Scientific Research and Development to the Bureau of Entomology and Plant Quarantine.

[2] Now with Cornell University. The writer wishes to acknowledge the assistance of many of the staff members at Orlando, Fla., and at Portland, Oreg., in making these tests. Special acknowledgement is due Lyda Roberson, who assembled most of the records for this report, and Helen Fluno, who prepared the chemical names to conform with the Chemical Abstracts system.

TABLE 1

Results of tests with insect repellents most effective against Anopheles quadrimaculatus
Orlando, Florida, 1942–1945

REPELLENTS	TESTS	AVERAGE REPELLENT TIME
	Number	*Minutes*
Test materials		
Acetamide, *N*,*N*-diamyl-	28	122
Acetamide, *N*,*N*-diisoamyl-	18	149
Acetic acid, chloro-, 2-nitroisobutyl ester	14	158
Acetic acid, cyano-, cyclohexyl ester	8	129
Acetophenone, *p*-ethoxy-	27	123
Anthranilic acid, methyl ester	20	163
Benzaldehyde, *o*-ethoxy-	20	126
Benzaldehyde, *p*-isopropoxy-	8	180
Benzoic acid, *o*-ethoxy-, methyl ester	6	136
Benzyl alcohol, *p*-ethoxy-	13	134
Benzyl alcohol, *o*-methoxy-	24	174
Caproic acid, 2-ethyl-	22	137
Caproic acid, tetrahydrofurfuryl ester	11	127
Caprylic acid, tetrahydrofurfuryl ester	2	145
Cinnamaldehyde, alpha-amyl-	38	232
Crotonic acid, beta-diethylamino-, methyl ester	8	189
1-Cyclohexen-1-acetic acid, 2-hydroxy-, lactone mixture with $\Delta^{1,\ alpha}$-cyclohexaneacetic acid, 2-hydroxy-, lactone	6	173
2-Cyclohexene-1-ol, 1-phenyl-	4	128
1,1-Cyclopropanedicarboxylic acid, dibutyl ester	7	140
Glycine, *N*-butyl-, isobornyl ester	12	150
Isovaleric acid, diester with 1,2-propanediol	2	123
Mesitylene, 2-bromo-	4	126
Oil, geranium	1	140
1-Oxaspiro[2,5]octane-2-carboxylic acid, 2-methyl-, allyl ester	8	126
Phenethyl alcohol, *o*-methyl-	18	149
Phenol, tris(ethylaminomethyl)-	12	159
Propionic acid, diester with 1,2-hexanediol	4	126
Succinic acid, alpha-cyano-beta-methyl-, diethyl ester	35	145
Succinimide, *N*-amyl-	29	208
Wood-tar distillate	21	159
Standard repellents		
1,3-Hexanediol, 2-ethyl- (Repellent 6-12)	154	53
Phthalic acid dimethyl ester (dimethyl phthalate)	3,512	108
1,2*H*-Pyran-6-carboxylic acid, 3,4-dihydro-2,2-dimethyl-4-oxo-, butyl ester (Indalone)	76	41
Mixture 6-2-2 (6 parts dimethyl phthalate and 2 parts each Repellent 6-12 and Indalone)	179	147

RESULTS

Anopheles quadrimaculatus (table 1). Of 4,313 repellents tested, 2,031 were effective for 0 to 30 minutes; 1,734 for 31 to 60 minutes; 406 for 61 to 90 minutes; and 142 for 91 or more minutes. Results with the standard repellents dimethyl phthalate, Re-

pellent 6-12, Indalone, and a 6-2-2 mixture of these materials and with 30 materials that gave more than 2 hours' repellent time are presented in Table 1.

The following were effective for 91 to 120 minutes:

Acetamide, alpha-(2-butoxyethoxy)-*N*-cyclohexyl-
Acetamide, *N*,*N*-dibutyl-
Acetamide, *N*,*N*-di-*sec*-butyl-
Acetanilide, *N*-butyl-
Acetic acid, decyl ester
Acetic acid, *o*-phenylenedioxy-, methyl ester
Acetic acid, thiocyano-, fenchyl and isobornyl esters (Thanite)
2-Acetonaphthone, 5,6,7,8-tetrahydro-
Acetophenone, *p*-methoxy-
Adipic acid, diisobutyl ester
Adipic acid, beta-methyl-, diethyl ester
Anthranilic acid, *tert*-butyl ester
Anthranilic acid, isoamyl ester
Anthranilic acid, propyl ester
Anthranilic acid, *N*-methyl-, methyl ester
Benzaldehyde, *o*-allyloxy-
Benzaldehyde, *p*-butoxy-
Benzaldehyde, *p*-propoxy-
Benzene, 2-bromo-1,3,5-triethyl-
Benzoic acid, hexyl ester
Benzoic acid, *o*-methoxy-, methyl ester
Bibenzyl, alpha-methyl-
Butyraldehyde, alpha-(2-cyanoethyl)-alpha-ethyl-
Butyric acid, diester with 2-methyl-1,3-propanediol
Butyric acid, diester with 1,5-pentanediol
Butyric acid, 2-phenoxyethyl ester
Butyrophenone, *p*-chloro-
Butyrophenone, *p*-methoxy-
Camphane, 2-(2-amino-3-hydroxy-2-methylpropoxy)-
Caproic acid, amyl ester
Caproic acid, cyclohexyl ester
Caprylaldehyde
Caprylic acid, alpha-hydroxy-, butyl ester
Carbamic acid, acetyl(octyloxy)-, propyl ester
Carbonic acid, ethyl 2-phenylethyl ester
Cinnamic acid, allyl ester
Cinnamic acid, butyl ester
Cinnamic acid, *sec*-butyl ester
Cinnamic acid, *tert*-butyl ester
Cinnamic acid, ethyl ester
Cinnamic acid, isopropyl ester
Cinnamic acid, *o*-methoxy-, methyl ester, *cis*
Crotonic acid, diester with 2-methyl-1,3-propanediol
Cumic acid, methyl ester
Cumidine
$\Delta^{1,alpha}$-Cyclohexaneacetic acid, alpha-cyano-, methyl ester
Cyclohexanecarboxylic acid, 1-methyl-2-oxo-, ethyl ester
1,2-Cyclohexanedicarboxylic acid, dipropyl ester
Cyclopentanecarboxylic acid, 1-hydroxy-, isoamyl ester
5-*m*-Dioxanol, 2-hexyl-

1,3-Dioxolane-4-methanol, 2-(*p*-methoxyphenyl)-, plus 5-*m*-dioxanol, 2-(*p*-methoxyphenyl)-
1,3-Dioxolan-5-ol, 2-amyl-
Dodecanal
6-Dodecanone, 7-hydroxy-
Enanthic acid
Enanthic acid, heptyl ester
Ethanol, 2-(2-butoxyethoxy)-, acetate
Ethanol, 2-(*p*-*tert*-butylphenoxy)-
Ethanol, 2-(x,y-diethylphenoxy)-
Ethanol, 2-(x-ethylphenyl)-
Ethanol, 2-(2-hexyloxyethoxy)-
Ethanol, 2-(x-toloxy)-
Ether, benzyl cyclohexyl
Ether, 2-bromoethyl *p*-chlorophenyl
Ether, *p*-bromophenyl 3-chloroisobutyl
Ether, *p*-*tert*-butylphenyl 2-methylallyl
Ether, 2-chloroethyl *o*-cyclohexylphenyl
Ether, 3,4-methylenedioxybenzyl propyl
2-Furanacrylic acid, propyl ester
Furfuryl alcohol, alpha-butyl-, acetate
Glycidic acid, beta-propyl-beta-(2-thienyl)-, ethyl ester
Glyoxylic acid, phenyl-, methyl ester
Hendecenoic acid
4-Heptanol, 3-nitro-
1,6-Hexanediol, diacetate
3,4-Hexanediol, 3,4-diethyl-
Hydrocinnamic acid, propyl ester
Isobutyric acid, diester with 1,3-propanediol
Lactic acid, 2-(2-ethoxyethoxy)ethyl ester, acetate
Lactic acid, methylallyl ester, acetate
Lauric acid, 2-chloroallyl ester
Malonic acid, bromo-, diethyl ester
Malonic acid, isopropyl(1-pentenyl)-, diethyl ester
Malonic acid, (1-propenyl)propyl-, diethyl ester
Mesitylenaldehyde
2-Naphthol, decahydro-
2-Naphthol, 1,2,3,4-tetrahydro-
Pelargonic acid, theta-hydroxy-, lactone
2-Pentenoic acid, 2-cyano-3-ethyl-, ethyl ester
4-Pentenophenone
Phenethyl alcohol, alpha-isopropyl-
Phenethyl alcohol, *m*-methyl-
Phenetole, x-*tert*-butyl-
Phenol, 4-chloro-2-phenyl-, acetate
Phenol, 2-phenylethyl-
Phthalic acid, dimethyl ester
Pinacolone, furfurylidene-
Pivalic acid, 3-butynyl ester
Resorcinol, monoacetate
Succinic acid, diisoamyl ester
Succinic acid, dipropyl ester
2,4,8,10-Tetroxaspiro [5.5]hendecane, 3,9-diethyl-
Toluene, dibromo-
Tolyl ether

Tributenylamine
Triethylene glycol, terpinyl ether*
Valeric acid, phenylethyl ester
Velsicol AR-40 (an aromatic petroleum solvent)
Wood, distillates from, 66 fraction, chloroacetic acid ester*
Wood, distillates from, 68 fraction, chloroacetic acid ester*
Wood, distillates from, 68 fraction, methyl ether*
Wood-tar distillate*

Only 4 repellents, *p*-isopropoxybenzaldehyde, alpha-amylcinnamaldehyde, methyl beta-diethylaminocrotonate, and *N*-amylsuccinimide were effective for as long as three hours. Of the standard repellents, only dimethyl phthalate and the 6-2-2 mixture were sufficiently effective to be included with the better repellents.

Numerous tests were made with various formulations of the better repellents, especially dimethyl phthalate, in an effort to extend the repellent time. These formulations included the repellent in various thickeners, or mixed with other repellents, astringents, acids, alkaline materials, and insecticides, particularly pyrethrum. The repellents were also incorporated into creams and powder pastes. With the possible exception of the powder pastes, no encouraging results were obtained with any of the formulations.

Anopheles freeborni (table 2). Of 49 materials tested 8 were effective for more than 7 hours. Of the standard repellents dimethyl phthalate and Repellent 6–12 were included in this top group. Indalone was the least effective, and mixture 6-2-2, with an average protection time of 416 minutes, just missed being included with the best materials.

Anopheles punctipennis (table 2). Out of 12 materials tested against this species 5 were effective for more than 7 hours.

Anopheles albimanus (table 3). Field and laboratory tests were made in Panama by D. M. Jobbins with several materials submitted to him by the Orlando laboratory. The laboratory tests were made against *A. albimanus* Wied. and the field tests against a mixed population, predominantly *A. albimanus*, *A. punctimaculata* D. and K., *Mansonia titillans* (Walkr.), *M. nigricans* Coq.; a few *Culex* spp. were also present. In the laboratory a standard amount of material (1/4 teaspoonful) was distributed on the arms of test subjects, which were then exposed in cages containing several hundred mosquitoes, or cages containing 20 unfed mosquitoes were fastened to the treated arms. In the field the repellents were applied with small squares of gauze to the hands, arms, face, and neck of test subjects. The tests were made on persons who were perspiring freely.

The laboratory tests showed that Indalone lost its repellency in less than 40 minutes and dimethyl phthalate in 40 to 60 minutes. Repellent 6–12 was effective more than 80 to 100 minutes. The results of 18 field tests show that Repellent 6–12 and dimethyl phthalate were equally effective for the first hour, but that Repellent 6–12 was decidedly superior by the end of the second and third hours. Indalone was not effective for even one hour.

Anopheles species in Africa. The effectiveness of standard repellents used in tests

* Indicates that this is the best name available for this material.

TABLE 2

Results of tests with insect repellents against Anopheles freeborni *and* A. punctipennis, *Portland, Oregon, 1945*

REPELLENTS	TESTS	AVERAGE REPELLENT TIME
	Number	*Minutes*
Anopheles freeborni		
Acetic acid, 2-phenoxyethyl ester	4	392
Acetoacetic acid, cyclohexyl ester	4	256
Anisyl alcohol	7	223
Anthranilic acid, propyl ester	7	231
Benzaldehyde, 3,4-diethoxy-	6	250
Benzaldehyde, *o*-ethoxy-	3	267
Benzoic acid, benzyl ester	1	105
Benzoic acid, cyclohexyl ester	6	151
Benzoic acid, tetrahydrofurfuryl ester	5	227
Benzyl alcohol, *o*-methoxy-	4	450
Benzyl ether	4	80
Bicyclo[2.2.1]-5-heptene-2,3-dicarboxylic acid, diethyl ester	6	185
Bicyclo[2.2.1]-5-heptene-2,3-dicarboxylic acid, dimethyl ester, *cis*-	4	285+
Cyclohexaneacetic acid, alpha-cyano-, ethyl ester	4	73
Cyclohexanecarboxylic acid, 1-hydroxy-, cyclopentyl ester	4	66
1,2-Cyclohexanedicarboxylic acid, diethyl ester	5	240
Cyclohexanol, 2-phenyl-	6	289
m-Dioxane, 2-hexyl-4(or 5)-methoxy-	5	173
m-Dioxane, 4-(*p*-methoxyphenyl)-5-methyl-	5	74
5-*m*-Dioxanol, 2-hexyl-	5	363
1,5-Dioxaspiro[5.5]-hendecan-3-ol, 11-methyl-	5	440+
1,3-Dioxolane, 5-methyl-5-nitro-2-propyl-	5	145
Ethanol, 2-(alkylbenzyloxy)-	4	51
Ethanol, 2-(2-butoxyethoxy)-, acetate	7	289
Ethanol, 2,2′-thiodi-, diacetate	5	81
Hendecenoic acid	5	445
1,3-Hexanediol, 2-ethyl-	15	466
Hydracrylic acid, beta-phenyl-, ethyl ester	5	258
Hydrocinnamic acid, alpha,beta-epoxy-beta-methyl-, ethyl ester	5	64
Isobutyl sulfone	4	378
Isobutyric acid, alpha-hydroxy-, 2-phenethyl ester	4	392+
dl-Malic acid, dibutyl ester	5	183
2-Naphthol, 1,2,3,4-tetrahydro-	5	452+
3-Octyne-2,5-diol, 2,5,7-trimethyl-	4	100
Phenethyl alcohol, *p*-isopropyl-	5	453+
Phthalic acid, diallyl ester	4	45
Phthalic acid, dibutyl ester	2	54
Phthalic acid, dimethyl ester	16	447
Phthalimide, *N*-*sec*-butyl-	5	119
Phthalimide, *N*-butyl-1,2,3,6-tetrahydro-	6	106
1,2-Propanediol, 3-(1,3-dimethylbutoxy)-	5	354
1-Propanol, 3-(3-cyclohexyloxypropoxy)-	4	331
Propionic acid, diester with 1,5-pentanediol	4	249

TABLE 2—*Continued*

REPELLENTS	TESTS	AVERAGE REPELLENT TIME
	Number	*Minutes*
Anopheles freeborni—Continued		
1,2*H*-Pyran-6-carboxylic acid, 3,4-dihydro-2,2-dimethyl-4-oxo-, butyl ester (Indalone)	13	274
Repellent mixture (80% dimethyl phthalate plus 20% 2-ethyl-1,3-hexanediol)	7	438+
Mixture 6-2-2 (6 parts of dimethyl phthalate and 2 parts each of Repellent 6-12 and Indalone)	4	416
Succinamic acid, *N*,*N*-diethyl, propyl ester	5	213
Succinamic acid, *N*,*N*-dipropyl-, ethyl ester	6	153
Succinic acid, alpha-cyano-beta-methyl-, diethyl ester	4	31
Anopheles punctipennis		
Anthranilic acid, propyl ester	3	197+
Benzoic acid, benzyl ester	7	255
Benzoic acid, cyclohexyl ester	4	300
Cyclohexanol, 2-phenyl-	2	458+
Ethanol, 2-(2-butoxyethoxy)-, acetate	12	341
1,3-Hexanediol, 2-ethyl-	17	477
Phthalic acid, diallyl ester	2	60
Phthalic acid, dibutyl ester	7	169
Phthalic acid, dimethyl ester	17	447
1,2*H*-Pyran-6-carboxylic acid, 3,4-dihydro-2,2-dimethyl-4-oxo-, butyl ester (Indalone)	18	282
Repellent mixture (80% dimethyl phthalate plus 20% 2-ethyl-1,3-hexanediol)	2	490+
Mixture 6-2-2	17	455

TABLE 3

Number of bites received per person from a mixed population of mosquitoes, primarily Anopheles albimanus, *after treatment with standard repellents. Average of 18 field tests. Panama 1942.*

REPELLENT	AFTER FIRST HOUR	AFTER SECOND HOUR	AFTER THIRD HOUR
Repellent 6-12	0.2	0.4	0.8
Dimethyl phthalate	.2	3.2	10.2
Indalone	5.2	15.8	27.9

made against *Anopheles g. gambiae* Giles in Africa was reported to us by Major Lewis Berner, Sanitary Corps, U. S. Army, as being of short duration. The tests were made by the procedure described in this paper. The average repellent times for dimethyl phthalate, Indalone, and Repellent 6–12 was only 45, 30, and 20 minutes, respectively.

Major Berner also reported on field tests in Africa against a mixed population of *Anopheles*, principally *A. gambiae melas* Theob. In paired tests with six repellents and mixture 6-2-2 he found that only dibutyl malate was inferior to the mixture (table 4). Major Berner's letter states: "All of the repellents were very effective against *Anopheles gambiae gambiae* Giles, *A. gambiae melas* Theo., *A. funestus funestus*

Giles, *A. nili* (Theo.), and *A. welcomei* Theo., and could be expected to furnish protection against these species for a minimum period of four (4) hours without reapplication. . . . 6-2-2 applied every two (2) hours to the skin of the writer and the sergeant assisting him gave complete protection from all species of *Anopheles* studied as well as culicines."

Anopheles aquasalis Curry. In 1943 field tests against a population consisting primarily of *A. aquasalis* were made in Trinidad by the late R. C. Shannon (personal communication). Some *Mansonia titillans* and *Culex* spp. were also present. The arms of the test subjects were smeared with the various repellents at different intervals

TABLE 4

Relative effectiveness of six repellents paired with mixture 6-2-2 against Anopheles gambiae melas. *Africa, 1945.*

REPELLENT	TIME TO FIRST BITE WITH	
	Test repellent	Mixture 6-2-2
Benzyl ether	395	299
Cyclohexanol, 2-phenyl-	320	242
Isobutyric acid, alpha-hydroxy-, 2-phenethyl ester	No bites	480
dl-Malic acid, dibutyl ester (Dibutyl malate)	237	389
Phthalimide, *N-sec*-butyl-	430	274
Succinimide, *N*-amyl-	No bites	232

TABLE 5

Average number of bites per person received from a mixed population of mosquitoes, primarily Anopheles aquasalis. *After treatment with standard repellents. Trinidad, 1943.*

MATERIAL	2½ HOURS	4 HOURS	6 HOURS	8 HOURS
Indalone	3	26	22	8
Repellent 6-12	1	4	36	10
Dimethyl phthalate[1]	3	28	1	—
Untreated subjects	73	108	139	75

[1] Tests with this material were made 30 minutes later than given in the table.

before the subjects were exposed for $1\frac{1}{4}$ hours in an area where the mosquitoes were abundant.

The results (table 5) were somewhat variable, but Indalone, dimethyl phthalate, and Repellent 6–12 all gave good protection for $2\frac{1}{2}$ hours after treatment. After 4 hours, Repellent 6–12 was the most effective material and Indalone and dimethyl phthalate were about equal. Even after 8 hours subjects treated with Indalone and Repellent 6–12 were receiving fewer bites than untreated subjects.

SUMMARY

Results of field and laboratory tests with several repellents against different species of *Anopheles* are summarized. Of 4,313 compounds tested against *Anopheles quadrimaculatus* Say, the 142 most effective compounds are listed, and detailed results are

given for the 30 most effective. Only 4 materials, *p*-isopropoxybenzaldehyde, alpha-amylcinnamaldehyde, methyl beta-diethylaminocrotonate, and *N*-amylsuccinimide were effective for as long as 3 hours. Of the standard repellents only dimethyl phthalate and the 6-2-2 mixture (dimethyl phthalate, Repellent 6–12, and Indalone) were sufficiently effective to be included with the best repellents. Indalone and Repellent 6–12 averaged less than one hour of repellent time. *Anopheles freeborni* Aitken and *A. punctipennis* (Say) were more easily repelled, 8 of 49 materials and 5 of 12 materials being effective for more than 7 hours against these species, respectively. Against *Anopheles albimanus* Wied. the materials showed repellencies similar to those against *A. quadrimaculatus*, except that Repellent 6–12 was superior to dimethyl phthalate. In cage tests *Anopheles gambiae* Giles were repelled for less than an hour with dimethyl phthalate, Repellent 6–12, and Indalone. In the field several materials were more effective than the 6-2-2 mixture. Against *Anopheles aquasalis* Curry, Indalone, dimethyl phthalate, and Repellent 6–12 gave good protection for 2½ hours after treatment and partial protection after 8 hours.

RESUMEN

Aparecen compendiados los resultados de experimentos llevados a cabo en el laboratorio y en el campo utilizando repelentes contra diferentes especies de *Anófeles*. Aparecen catalogados los 142 compuestos más efectivos entre los 4,313 probados contra *Anopheles quadrimaculatus* Say y preséntanse resultados detallados de los 30 compuestos más efectivos. Solamente cuatro materiales, "*p*-isopropoxybenzaldehyde", "alpha-amylcinnamaldehyde", "methyl beta-diethylaminocrotonate" y "*N*-amyl-succinimide" resultaron efectivos durante tres horas. De los compuestos clásicos, solamente "dimethyl phthalate" y la mezcla 6-2-2 ("dimethyl phthalate", Repelente 6-12 e "Indalone") resultaron suficientemente efectivos para ser considerados entre los mejores repelentes. "Indalone" y Repelente 6-12 promediaron menos de una hora de tiempo repelente. *Anopheles freeborni* Aitken y *Anopheles punctipennis* Say fueron más fácilmente rechazados, 8 de 49 materiales y 5 de 12 resultando efectivos por más de 7 horas contra las respectivas especies. Los materiales demostraron poder repelente contra *Anopheles albimanus* similar al obtenido contra *A. quadrimaculatus* excepto que Repelente 6-12 resultó superior a dimethyl phthalate. En experimentos llevados a cabo en jaulas *Anopheles gambiae* Giles fué rechazado durante menos de una hora por "dimethyl phthalate", Repelente 6-12 e "Indalone". En el campo varios materiales resultaron más efectivos que la mezcla 6-2-2. "Indalone", "dimethyl phthalate" y Repelente 6-12 demostraron gran protección contra *Anopheles aquasalis* Curry por dos horas y media después de la aplicación y protección parcial durante ocho horas.

MOSQUITO REPELLENTS FOR APPLICATION TO CLOTHING[1]

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Laboratory and field tests were conducted during 1948 and 1949 at the Orlando Fla., laboratory of the Bureau of Entomology and Plant Quarantine to select suitable repellents to be applied to clothing for protection of military personnel against various species of mosquitoes. Materials were sought that would remain effective for a considerable period after the clothing had been treated. In previous studies to select more effective repellents (Smith and Burnett, 1949), several thousand compounds were screened against *Aedes aegypti* (L.). Evaluation was on the basis of aging-tests, conducted in the laboratory, using cotton stockings impregnated at the rate of 3 grams per square foot of cloth. Compounds effective for 10 days or more were given further aging-tests in the field against the salt-marsh mosquitoes *Aedes taeniorhynchus* (Wied.) and *A. sollicitans* (Walk.)

The present study consisted of more critical tests to eliminate all but the most durable repellents. All treatments except those with aerosols were made by impregnating the clothing with acetone solutions or emulsions of the repellents. The concentration of the solutions or emulsions was adjusted to provide the desired dosage of the repellent in the minimal amount of liquid required to saturate the cloth. The volume of liquid used was 75 cc. for single stockings, which measured about 2.2 square feet of cloth, and 1,500 cc. for entire fatigue uniforms, which averaged about 37 square feet of cloth.

WEARING TESTS USING INDIVIDUAL REPELLENTS

A total of 151 chemicals found most effective in the aging-tests were subjected to wearing-tests against the bites of salt-marsh mosquitoes in the field. Stockings were worn by laboratory personnel after impregnation with repellents dissolved in acetone, in amounts of two and three grams per square foot. These were worn for 8-hour periods. They were then tested in the field for five minutes each on three different persons. When the average number of bites through the stockings was less than five in five minutes the repellent was considered effective. Compounds were eliminated if they (1) caused irritation to the skin of test subjects, (2) were reported to be unsafe for use according to toxicological tests on rabbits by the Food and Drug Administration, (3) stained fabrics, or (4) had a very strong odor.

Results with 11 of the best materials thought to be safe are presented in table 1. Three standard mosquito repellents, for skin application, and an effective tick repellent, for treatment of clothing, are included for comparison. Methyl *N*,*N*-diisopropyladipamate and 2-butyl-2-ethyl-1,3-propanediol were effective at 3 grams per

[1] This work was conducted under funds allotted by the Department of Defense to the Bureau of Entomology and Plant Quarantine.

[2] The authors acknowledge the assistance of Dr. W. V. King in planning the tests, and of other members of the staff in the testing.

square foot after 56 hours of wear in one test, and *N*-butyl-1,2,3,6-tetrahydrophthalimide, Indalone, and undecylenic acid (hendecenoic acid) were effective after 48 hours. Undecylenic acid was dropped after the first test because its odor was somewhat objectionable and other equally effective materials were at hand. It is included here because it was later found to be unequaled as a flea repellent and may be selected for use in an all-purpose mixture.

TABLE 1

Duration of protection against salt-marsh mosquitoes afforded by various repellents in tests using impregnated stockings

REPELLENT	HOURS OF WEAR WITEST OOD AT INDICATED DOSAGE PER SQU ARE FOOT			
	3 grams		2 grams	
	Series 1	Series 2	eries 1	Series 2
Methyl *N*,*N*-diisopropyladipamate	56	48	48	16
2-Butyl-2-ethyl-1,3-propanediol	56	40	32	16+[1]
N-Butyl-1,2,3,6-tetrahydrophthalimide	48	32	40	24
Indalone	48	24	16	8
Hendecenoic acid (undecylenic acid)	48		—	—
N,*N*-Dibutylacetoacetamide	32	24	24	8
Propyl *N*,*N*-diethyl succinamate	24	16	40	16
Hexyl mandelate	40	16+	<8	<8
N-Isopropylacetanilide	24	16	24	8
2,4-Nonanediol	24	16	24+	8
Ethyl beta-phenylhydracrylate	24	16	8	8
Dimethyl phthalate	16		—	—
N-Butylacetanilide	8		—	—
Repellent 6-12	<8		—	—
6-2-2 Mixture (dimethyl phthalate 60%, Indalone 20%, Repellent 6-12 20%)	<8		—	—

[1] Plus signs indicate records terminated while the repellent was still effective.

FIELD TESTS WITH REPELLENTS IN ALL-PURPOSE MIXTURES

On the basis of these tests 2-butyl-2-ethyl-1,3-propanediol and Indalone were selected for inclusion in mixtures with the best tick, flea, and chigger repellents to provide one all-purpose clothing treatment. Methyl *N*,*N*-diisopropyladipamate had not been cleared toxicologically at the time, and Indalone was given precedence over *N*-butyl-1,2,3,6-tetrahydrophthalimide because it was also one of the better tick repellents. *N*-Butylacetanilide was included as an outstanding repellent against ticks and oriental rat fleas, diphenyl carbonate as a chigger repellent, and benzyl benzoate as a cat flea and chigger repellent. (Since the conclusion of these experiments toxicological studies by the Army Environmental Hygiene Laboratory have shown diphenyl carbonate to be unacceptable as a clothing treatment; other materials listed herein may later prove undesirable.)

T-shirts and herringbone twill trousers were treated with emulsions of 16 mixtures and subjected to wearing tests against salt-marsh mosquitoes. As in the previous tests,

these garments were worn for eight hours between tests. On T-shirts most of the mixtures were effective until the shirts were too badly soiled for further wear, in most cases for 24 hours. On trousers the duration of effectiveness ranged from less than eight to more than 40 hours.

TABLE 2

Effectiveness of repellents, applied as emulsions to fatigue uniforms, against salt-marsh mosquitoes

MIXTURE NO.	CHEMICAL	PER CENT[1]	GRAMS PER SQUARE FOOT	AVERAGE PER CENT REPELLENCY	
				Series 1	Series 2
	Mixtures				
M-1960	2-Butyl-2-ethyl-1,3-propanediol	30	1.07		
	N-Butylacetanilide	30	1.07	99	96
	Benzyl benzoate	30	1.07		
M-1981	2-Butyl-2-ethyl-1,3-propanediol	25	1.33		
	N-Butylacetanilide	25	1.33	96	99
	Diphenyl carbonate	10	0.53		
	Xylene	30	—		
M-1980	2-Butyl-2-ethyl-1,3-propanediol	20	1.07		
	Indalone	30	1.60	97	96
	Diphenyl carbonate	10	0.53		
	Xylene	30	—		
M-1979	*N*-Butylacetanilide	35	1.00		
	Diphenyl carbonate	17.5	0.50	94	93
	Xylene	37.5	—		
M-1978	Indalone	60	2.00		
	Benzyl benzoate	30	1.00	83	93
M-1962	*N*-Butylacetanilide	45	1.00		
	Benzyl benzoate	45	1.00	80	89
	Single repellents				
	2-Butyl-2-ethyl-1,3-propanediol	45	2.00	92	91
	Xylene	45			
	N-Butylacetanilide	90	2.00	87	96
	Indalone	90	2.00	95	87

[1] All concentrates contained 10 per cent of Tween 80 as emulsifier.

Six of the most effective mixtures were then subjected to further evaluation using complete fatigue uniforms made of herringbone twill. Uniforms treated with 2-butyl-2-ethyl-1,3-propanediol, Indalone, or *N*-butylacetanilide were included as standards of comparison. All test agents were applied as emulsions.

Tests were made on the 2nd, 4th, 6th, and 8th days after treatment with 16 hours

of intervening wear. Exposure to mosquitoes was for 10 minutes in each of three locations. Repellency was calculated from differences in the number of bites received by wearers of treated and untreated uniforms. The number of bites through untreated uniforms ranged from 71 to 364 (average 194) during the 30-minute exposure. Averages of the results obtained in two series of tests are shown in table 2. Three mixtures containing 2-butyl-2-ethyl-1,3-propanediol were more than 95 per cent effective. Of the single compounds tested for comparison, Indalone was best in one series and *N*-butylacetanilide in another, but 2-butyl-2-ethyl-1,3-propanediol was second in both and was the only individual repellent consistently more than 90 per cent effective.

TABLE 3

Duration of repellency of various mixtures and individual repellents against Aedes aegypti *and* Anopheles quadrimaculatus, *in laboratory tests with impregnated stockings*

Mixtures applied at dosages given in table 2, others at 2 grams per square foot. Average of duplicate tests.

REPELLENT[1]	DAYS' EFFECTIVE AGAINST			
	A. quadrimaculatus		*A. aegypti*	
	Emulsion	Solution	Emulsion	Solution
M-1960	30	—	31	—
M-1981	22	—	22	—
2,4-Nonanediol	—	17	—	20
Dimethyl phthalate	16	12	9	11
6-2-2 mixture	16	9	9	9
N,*N*-Dibutylacetoacetamide	—	14	—	13
M-1980	12	—	16	—
Repellent 6-12	8	13	10	12
N-Butyl-1,2,3,6-tetrahydrophthalimide	8	12	20	31
2-Butyl-2-ethyl-1,3-propanediol	8	11	8	22
N-Butylacetanilide	9	8	13	9
M-1962	6	—	7	—
Hexyl mandelate	2	0	2	0
M-1979	1	—	7	—
Methyl *N*,*N*-diisopropyladipamate	1	0	9	5

[1] See table 2 for composition of mixtures.

LABORATORY TESTS COMPARING MIXTURES WITH SINGLE REPELLENTS

Laboratory tests against *Anopheles quadrimaculatus* Say and *Aedes aegypti* were conducted in order to compare certain mixtures with the best of the individual repellents from field tests (table 1). Standard repellents for skin application were also included for comparison. In the first series of tests the standard laboratory procedure of exposing an impregnated cotton stocking on a subject's arm to caged mosquitoes after various intervals of aging, was followed. When less than five bites were received in one minute, the treatment was considered effective. Both emulsions and acetone solutions were employed in the impregnations at concentrations as required to give the desired dosages. The results are presented in table 3.

The most effective agent against both species of mosquitoes was mixture M-1960. Another mixture, M-1981, was second in effectiveness against *Anopheles* and third against *Aedes aegypti*. The best individual compounds were 2,4-nonanediol against *Anopheles* and *N*-butyl-1,2,3,6-tetrahydrophthalimide against *Aedes aegypti*. Repellent 6–12, dimethyl phthalate, and the 6-2-2 mixture were much more effective in these tests than in the field tests against the salt-marsh species. There appeared to be no consistent difference between emulsions and acetone solutions containing the same repellent.

TABLE 4

Duration of repellency of various mixtures and individual repellents against Aedes aegypti *and* Anopheles quadrimaculatus, *in tests with impregnated stockings after repeated 8-hour periods of wear*

Averages of three tests; individual repellents and 6-2-2 mixture applied at 2 to 3 grams per square foot, other mixtures at 1 to 1½ times the dosage shown in table 2.

REPELLENT[1]	HOURS OF WEAR WITHSTOOD			
	A. quadrimaculatus		*A. aegypti*	
	Emulsion	Solution	Emulsion	Solution
M-1960	16	—	11	—
Dimethyl phthalate	13	13	8	5
M-1981	11	—	13	—
6-2-2 mixture	11	11	11	5
Repellent 6-12	8	13	11	13
M-1962	8	—	3	—
2-Butyl-2-ethyl-1,3-propanediol	5	8	8	5
N-Butylacetanilide	5	8	5	8
M-1979	5	—	5	—
N-Butyl-1,2,3,6-tetrahydrophthalimide	3	5	16	11
M-1980	3	—	16	—
Hexyl mandelate	0	0	3	8
Methyl *N*,*N*-diisopropyladipamate	0	0	3	3

[1] See table 2 for composition of mixtures.

In another series of experiments against the same species of mosquitoes, treated stockings were worn for eight hours between tests, the effectiveness was based on the hours of wear withstood without loss of the minimum required repellency. The results are presented in table 4. Here again, M-1960 was the most effective against *Anopheles quadrimaculatus* and one of the most effective against *Aedes aegypti*. Mixture M-1980 and *N*-butyl-1,2,3,6-tetrahydrophthalimide were the most effective against *A. aegypti* but among the least effective against *A. quadrimaculatus*. Dimethyl phthalate, M-1981, the 6-2-2 mixture, and Repellent 6-12 were next in effectiveness against *A. quadrimaculatus*, and all except dimethyl phthalate were also among the most effective against *A. aegypti*.

On the basis of these tests and tests against other species, mixture M-1960 was selected for an all-purpose repellent treatment. The mixtures containing diphenyl

carbonate are not being recommended because of the subsequent unfavorable toxicological reports.

COMPARISON OF ACETONE SOLUTIONS AND AEROSOLS

Four repellents were applied to stockings in acetone solution by the usual method of impregnation, and sprayed on other stockings as aerosols. After each eight hours of wear the effectiveness of the repellents was determined against *Aedes aegypti* in the laboratory. In another series of tests, stockings were similarly treated and tested after various periods of aging without wear. In both series of tests the dosages ranged from 2 to 3 grams per square foot.

Three of the aerosol formulas contained 20 per cent of Indalone, dimethyl carbate, or ethyl beta-phenylhydracrylate, each with 40 per cent of methylene chloride and 40 per cent of Freon-12. The fourth formula contained 5 per cent each of 2-butyl-2-ethyl-1,3-propanediol and Velsicol AR-50 (chiefly mono and dimethylnaphthalenes), 45 per cent each of methylene chloride, and Freon-12.

All the aerosol treatments were equal to or slightly better than those made with acetone solutions. None of the four repellents was very resistant to wear, but ethyl beta-phenylhydracrylate remained effective after 33 days of aging and dimethyl carbate after 19 to 26 days.

SUMMARY

Laboratory and field tests were conducted to select suitable repellents to be applied to clothing for protection of military personnel against various species of mosquitoes. Materials were sought that would remain effective in the clothing after several days' wear or several weeks' storage. Tests against the salt-marsh mosquitoes *Aedes taeniorhynchus* (Wied.) and *A. sollicitans* (Walk.) were conducted in the field. Those against *Aedes aegypti* (L.) and *Anopheles quadrimaculatus* Say were conducted in the laboratory.

Against salt-marsh mosquitoes the most effective repellents were methyl *N*,*N*-diisopropyl adipamate, 2-butyl-2-ethyl-1,3-propanediol, *N*-butyl-1,2,3,6-tetrahydrophthalimide, Indalone, and undecylenic (hendecenoic) acid. The outstanding individual repellents against *Aedes aegypti* or *Anopheles quadrimaculatus* or both were 2,4-nonanediol, *N*-butyl-1,2,3,6-tetrahydrophthalimide, 2-butyl-2-ethyl-1,3-propanediol, dimethyl phthalate, and Repellent 6-12.

Some of the most effective mosquito repellents were tested in mixtures containing outstanding tick, flea, and chigger repellents as all-purpose repellents. The most effective mixture against the three types of mosquitoes was M-1960. This mixture was also more effective against each species than any of the individual repellents.

Indalone, dimethyl carbate, and ethyl beta-phenylhydracrylate, when tested in aerosols, were equal to or slighly better than the same materials in acetone solution.

REFERENCE

SMITH, C. N., AND BURNETT, D., JR. 1949. Effectiveness of repellents applied to clothing for protection against salt-marsh mosquitoes. Jour. Econ. Ent., **42**: 43o–44.

RESUMEN

Se llevaron a cabo experimentos en el laboratorio y en el campo para seleccionar repelentes propios para ropa para lograr la protección de personal militar contra varias especies de mosquitos. Buscáronse materiales que se mantendrían eficaces después de usarse la ropa por varios días o almacenarse por varias semanas. Los experimentos contra los mosquitos de marisma *Aedes taeniorhynchus* (Wied.) and *A. sollicitans* (Walk.) se condujeron en el campo, aquellos contra *Aedes aegypti* (L.) y *Anopheles quadrimaculatus* Say se efectuaron en el laboratorio.

Los repelentes más efectivos contra los mosquitos de marisma fueron "methyl *N*,*N*-diisopropyladipamate", "2-butyl-2-ethyl-1,3-propanediol", "*N*-butyl-1,2,3,6,-tetrahydrophthalimide", "Indalone" y "undecylenic (hendecenoic) acid". Los repelentes individuales sobresalientes contra *Aedes aegypti* o *Anopheles quadrimaculatus* o contra ambos fueron "2,4-nonanediol", "*N*-butyl-1,2,3,6-tetrahydrophthalimide", "2-butyl-2-ethyl-1,3-propanediol", "dimethyl phthalate", "*N*,*N*-dibutylacetoacetamide" y Repelente 6-12.

Algunos de los repelentes contra mosquitos más efectivos fueron probados en mezclas compuestas de repelentes sobresalientes contra garrapatas, pulgas y ácaros denominados repelentes para todos usos. La mezcla más efectiva contra los tres tipos de mosquitos fué M-1960. Esta mezcla fué también más efectiva contra cada especie de mosquito que cualquiera de los repelentes individuales.

Cuando "Indalone", "dimethyl carbate", y "ethyl beta-phenylhydracrylate", fueron probados en aerosoles los resultados fueron iguales o un poco mejor que cuando se usaron estos mismos materiales en solución de acetona.

PYLORIC SPINES IN MOSQUITOES

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While examining the midgut of an *Aedes aegypti* mosquito for the presence of an avian malaria infection, a lining of small spines was observed on the inner surface of the pylorus, or ileo-colon, which is the funnel-shaped portion of the hindgut located

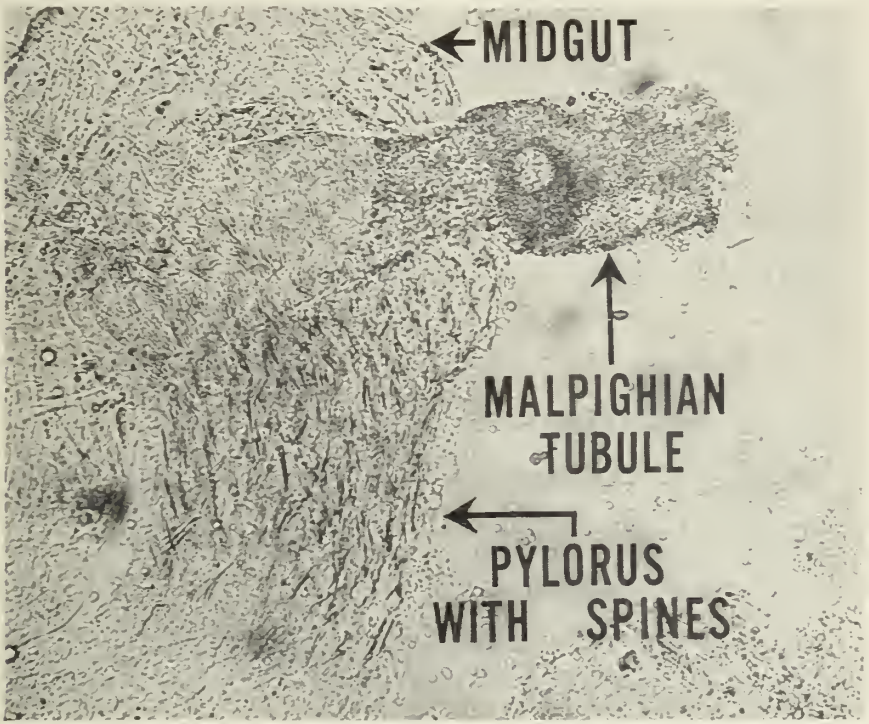

FIG. 1. Posterior portion of the alimentary canal of *Aedes aegypti* showing location of pyloric spines.

posterior to the attachment of the malpighian tubules. More than a dozen hindguts of *A. aegypti*, male and female, were then examined under the oil-immersion lens, and these spines were found in all. These structures are attached to the inner surface of the pylorus and directed posteriorly, with their long axes parallel to the length of the intestine. The spines, in groups of five to eight, are arranged in irregular rows. Those in the most anterior portion are fine and comblike, measuring approximately six microns in length; those more posterior are heavier, and measure about 16 microns in length.

The literature dealing with the internal anatomy of mosquitoes contains casual

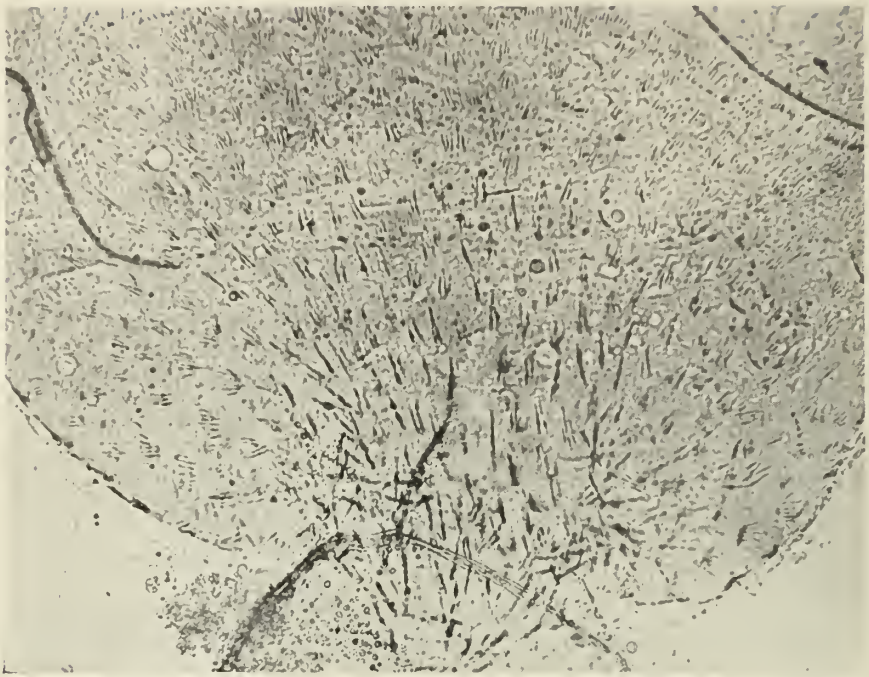

FIG. 2. A portion of the pylorus (×350) showing location and arrangement of the spines.

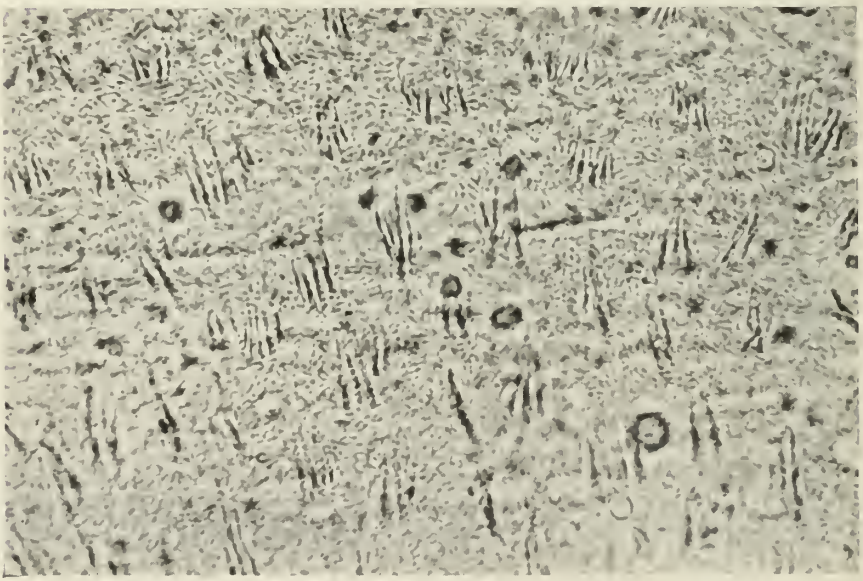

FIG. 3. A portion of the pylorus (×1200) showing morphology of the spines.

references to these spines in adult mosquitoes. Thompson (1905) mentions them as "bristle-like chitinous papillae" in Culex; Eysell (1905) describes "chitinous needles"

which "are directed toward the stomach" in Anopheles; de Boissezon (1930) refers to "bristling chitinous hairs" in *Culex pipiens;* and Richins (1938) remarks on the "rough spines" in *Aedes dorsalis*. When illustrated, they are depicted incidentally as single hairs or spines in drawings of longitudinal sections of that portion of the hindgut. Wigglesworth (1947) in a paragraph on insects possessing peritrophic membranes, states: "The cuticle of the hindgut frequently bears small backwardly directed spicules, which probably assist in drawing back the membrane and its contents when peristaltic waves pass along it."

I have found pyloric spines in both sexes of the following mosquito species: *Aedes aegypti*, *A. atropalpus*, *A. albopictus*, *A. triseriatus*, *Anopheles quadrimaculatus*, *A. freeborni*, *A. albimanus*, *A. aztecus*, *Culex pipiens*, and *C. quinquefasciatus*. There is a difference in size of the spines in Anopheles, Aedes, and Culex, the largest and most noticeable spines being present in *Aedes aegypti*, the smallest in *Anopheles aztecus*. The number, appearance, and arrangement of these spines differ with the genus, and superficially at least, there appear to be specific differences. These structures are of interest morphologically and physiologically; taxonomically, they may prove of value in separating closely related species.

Grateful acknowledgment is made to Mr. W. H. W. Komp and to Mr. R. Donald Reed of the National Institutes of Health, the former for his advice and criticism, and the latter for the photomicrographs which accompany this note.

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RESUMEN

Se ha llamado la atención a las pequeñas espinas que forran la superficie interior del píloro o íleo-colon del mosquito. Estas espinas se encontraron en ambos sexos de todas las especies examinadas: *Aedes aegypti*, *A. atropalpus*, *A. albopictus*, *A. triseriatus*, *Anopheles quadrimaculatus*, *A. freeborni*, *A. albimanus*, *A. aztecus*, *Culex pipiens* y *C. quinquefasciatus*. Se observaron diferencias genéricas y se notó la posibilidad de diferencias entre especies.

COMPARATIVE SUSCEPTIBILITY OF FOUR ANOPHELINE MOSQUITOES TO *PLASMODIUM RELICTUM*

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That avian malaria parasites can develop in anopheline mosquitoes has been shown by several workers. Recently, Hunninen *et al.* (1950) in a series of nine experiments fed *Anopheles quadrimaculatus* and *A. crucians* on English sparrows, *Passer domesticus*, infected with *Plasmodium relictum*. Forty-three and three tenths per cent of the *A. quadrimaculatus* and 11.1 per cent of *A. crucians* became infected. Complete development occurred in the *A. quadrimaculatus* but no sporozoites were found in the *A. crucians*. In another series of eight experiments the same species of mosquitoes were fed on canaries infected with *P. relictum* from wild-caught English sparrows; in these experiments only 1.9 per cent of the *A. quadrimaculatus* and none of the *A. crucians* developed the infection.

These results indicated that the susceptibility of other anopheline mosquitoes to avian malarias should be determined. The present investigation had the following objectives (1) to test the susceptibility of four anopheline species to *P. relictum* in English sparrows, (2) to determine whether the sporozoites would reach the salivary glands and in what numbers, and (3) to test the infectiveness of these sporozoites by injecting them into sparrows.

MATERIALS AND METHODS

The anopheline species used were *A. albimanus*, *A. quadrimaculatus*, *A. freeborni*, and *A. crucians*. *Culex pipiens* and *C. salinarius* were used as controls. The mosquitoes were obtained from laboratory colonies.

The *P. relictum* strains used were from two starlings (*Sturnus v. vulgaris*) and from three English sparrows which had the infection when captured. The starling strain was used in the first seven series of experiments, and the sparrow strain in the other eight experiments (see table 1). Sparrows 13, 187, and 288 were infected from starling 283. Sparrows 296, 240, and 426 received parasites from another starling not shown in table 1. The infection was passed from bird to bird by the intramuscular injection of infected citrated blood. Sparrow 22 was the original source of malaria for the birds represented in six of the series of experiments in the lower half of table 1. Sparrows 466 and 457 had original infections when trapped.

The various species of mosquitoes (with the exception of *A. freeborni*) were allowed to feed simultaneously on an infected bird placed in an insectary cage. Mosquitoes

[1] This investigation was supported (in part) by a research grant from the National Institutes of Health, Public Health Service.

[2] This work was carried out in the Laboratory of Tropical Diseases, U. S. Public Health Service, Columbia, South Carolina. I an indebted to Dr. Martin D. Young and Dr. Robert W. Burgess for their aid.

TABLE 1

Results of simultaneous feedings by different species of mosquitoes on birds infected with Plasmodium relictum

BIRD TYPE AND NUMBER	*Culex pipiens*						*Anopheles albimanus*						*Anopheles quadrimaculatus*						*Anopheles freeborni*						*Anopheles crucians*					
	Days after feeding	Number dissected	Infected				Days after feeding	Number dissected	Infected				Days after feeding	Number dissected	Infected				Days after feeding	Number dissected	Infected				Days after feeding	Number dissected	Infected			
			Guts	Glands	Total	Percent			Guts	Glands	Total	Percent			Guts	Glands	Total	Percent			Guts	Glands	Total	Percent			Guts	Glands	Total	Percent
Starling Strain																														
St.283*	21	13	2	2	2	15	21	2	0	0	0	0	21	29	0	0	0	0												
Sp.13	12	14	2	2	2	14							12	19	2	0	2	10												
Sp.187	21	7	7	7	7	100	21	3	3	3	3	100	21	15	0	0	0	0												
Sp.288	19	5	5	5	5	100							18	17	5	0	5	29												
Sp.296	18	8	8	8	8	100	18	12	11	12	12	100	18	35	15	3	15	43												
Sp.240	21	11	10	11	11	100	21	1	1	1	1	100	21	26	6	4	6	23												
Sp.426	14	10	10	10	10	100	14	25	24	13	24	96	14	18	0	0	0	0							19	50	5	0	5	10
Sparrow Strain																														
Sp.22*	20	6	6	6	6	100	20	2	2	2	2	100	20	15	8	3	8	53												
Sp.241	20	5	5	5	5	100							20	21	7	2	7	33												
Sp.404	12	13	13	13	13	100	12	14	14	5	14	100	12	14	13	12	13	93							18	17	6	1	6	35
Sp.411	18	7	7	7	7	100	18	8	6	4	6	75	18	6	6	4	6	100							18	24	7	3	7	29
Sp.452	14	2	2	2	2	100	14	7	7	5	7	100	14	11	8	6	8	73	14	18	13	8	13	72	14	10	4	1	4	40
Sp.450	13	10	10	10	10	100	13	13	10	7	10	77							13	14	4	5	5	35						
Sp.466*	14	6	5	5	5	83	14	5	1	1	1	20	14	9	0	0	0	0							14	2	1	1	1	50
Sp.457*	18	12	12	12	12	100	18	12	11	10	11	92	18	17	6	4	6	35												
Totals.....		129				87		104				80		252				35		32				54		103				33

Starling 283 and Sparrows 22, 466, and 457 had original infections when brought to the laboratory.

St.—Starling

Sp.—Sparrow

* Original infections in wild-caught birds

which did not feed were removed, but the engorged specimens were left in the cage and maintained at 76°F. ± 2°F. until dissected. To obviate possible errors in species identification *A. freeborni* and *A. quadrimaculatus* were fed in separate cages.

Since *P. relictum* develops more slowly in anophelines than in culicines, all dissections were made between the 12th and 21st day after feeding. This was sufficient time, in most cases, for the sporozoites to reach the salivary glands.

The techniques of staining blood smears and the care of infected mosquitoes have been described by Hunninen *et al.*, (1950).

RESULTS

Table 1 shows the results of simultaneous feedings by five different species of mosquitoes on birds infected with *P. relictum.* The most susceptible of the four species of anophelines used in these experiments was *A. albimanus*, 80 per cent of which became infected, compared with 87 per cent of *C. pipiens*, the control. In any single experiment, whenever the controls showed 100 per cent infection, the *A. albimanus* also

TABLE 2

Summary of infection rates in Culex pipiens *controls and in four anopheline species, following simultaneous feedings*

NUMBER OF SIMULTANEOUS FEEDINGS	*Culex pipiens* CONTROL MOSQUITOES, PERCENT INFECTED	EXPERIMENTAL MOSQUITOES	
		Species	Percent Infected
12	91.5	*A. albimanus*	80
14	86.5	*A. quadrimaculatus*	35
2	100.	*A. freeborni*	54
5	93.5	*A. crucians*	33

became 100 per cent infected or nearly so. Complete development of the parasites occurred in the *A. albimanus* with numerous salivary glands showing very heavy infections of sporozoites.

Of the 252 *A. quadrimaculatus*, 35 per cent became infected. Only 32 *A. freeborni* were dissected, but results indicate that this species is at least as susceptible as *A. quadrimaculatus*. The least susceptible of the anophelines was *A. crucians;* 33 per cent of 103 developed the infection. Complete development of the parasite occurred also in *A. quadrimaculatus*, *A. freeborni*, and *A. crucians*, many of which had salivary glands heavily infected with sporozoites.

It is of special interest that six *A. crucians* in four separate experiments showed sporozoites in their glands; of these, three had between 100 and 1,000 sporozoites, and one had more than 1,000. In our previous work (Hunninen *et al.*, 1950) complete development of *P. relictum* in *A. crucians* was not obtained.

In some of the experiments, sporozoites were found in the anophelines as early as the 12th day after feeding but in others development was slower, requiring from 17 to 20 days for completion.

The totals and percentages given in the last line of table 1 are the numbers of each species dissected, and the per cent found positive either with oocysts or sporozoites,

TABLE 3

Comparison of the Number of Oocysts in Mosquitoes that Fed on Birds With the Starling Strain of P. relictum *With the Number in Mosquitoes that Fed on Birds With the Sparrow Strain of* P. relictum

	Culex pipiens			*Culex salinarius*			*Anopheles albimanus*			*Anopheles quadrimaculatus*			*Anopheles freeborni*			*Anopheles crucians*		
	Number of Positives	Range in Number of Oocysts	Average Number of Oocysts	Number of Positives	Range in Number of Oocysts	Average Number of Oocysts	Number of Positives	Range in Number of Oocysts	Average Number of Oocysts	Number of Positives	Range in Number of Oocysts	Average Number of Cocysts	Number of Positives	Range in Number of Oocysts	Average Number of Oocysts	Number of Positives	Range in Number of Oocysts	Average Number of Oocysts
Starling Strain																		
Sp.13	2	7–20	64							2	2–4	3						
Sp.187	7	103–600	226				3	3–400	151									
Sp.288	5	28–83	53							5	1–40	12						
Sp.296	8	32–500	248				11	4–130	43	15	1–53	10						
Sp.240	10	27–500	245				1		72	6	1–95	36						
Sp.426	10	103–230	128				24	1–130	52							5	2–43	11
Average.....			161						80			15						11
Median.....			117						32			5						3
Sparrow Strain																		
Sp. 22	6	70–500	218				2	41–46	44	8	1–110	23						
Sp.241	5	130–250	201							7	1–15	5						
Sp.404	13	22–1500	571				14	26–1000	454	13	2–2000	848				6	1–800	187
Sp.411	7	Very many		9	1–400	162	6	8–1200	302	6	6–1000	491				7	1–18	8
Sp.452	2	1000–1400	1200	7	85–1200	667	7	48–340	141	8	4–1400	410	13	1–1000	123	4	1–500	134
Sp.450	10	77–1100	263				10	15–230	144				4	3–310	86			
Sp.466	5	12–800	260	11	6–1100	822	1		600							1		29
Sp.457	12	4–283	113				11	2–73	17	6	1–102	37						
Average.....			404			550			243			302			105			90
Median.....			170			285			119			30			5			16

Sparrows 13 to 426 infected with starling strain of *P. relictum*
Sparrows 22 to 457 infected with sparrow strain of *P. relictum*

or both. Table 2 summarizes the infection rates found after simultaneous feedings on infected birds by four anophe ine species and the *Culex pipiens* controls.

Mosquitoes that fed on sparrows having a strain of *P. relictum* obtained from sparrows showed more oocysts than did mosquitoes fed on sparrows infected with a strain from starlings. This is shown in table 3. The medians support the averages in each experiment. Individual variations in infectivity were noted, in that sparrows 404, 411, and 452 produced especially heavy infections in mosquitoes fed on them. Some of the *A. quadrimaculatus* were so heavily infected that the number of oocysts over 1,000 had to be estimated. Two *A. crucians* in this series showed 500 and 800 oocysts respectively.

TABLE 4

Results of inoculating sporozoites from three anopheline species and from two culicine species of mosquitoes into wild-caught English sparrows and into canaries

DEGREE OF INFECTION WITH E.E. BODIES	ENGLISH SPARROWS		CANARIES
	4 to 15 days after inoculation	16 to 18 days after inoculation	4 to 30 days after inoculation
Very heavy and heavy infections	21	0	0
Moderate No.	5	9	0
Few	9	6	0
Negative	3	0	11

TABLE 5

Results of the examination of 47 wild-caught English sparrows for erythrocytic and exoerythrocytic stages used for controls for inoculations shown in table 4

NEG. ERYTHROCYTES, NEG. FOR E.E. BODIES	POSITIVE ERYTHROCYTES NEG. FOR E.E. BODIES	NEG. ERYTHROCYTES. VERY FEW E.E. BODIES	POSITIVE ERYTHROCYTES, FEW E.E. BODIES	NEG. ERYTHROCYTES, MOD. NO. OF E.E. BODIES
29	3	11	3	1

In ten separate experiments, 53 wild-caught English sparrows and 11 canaries were injected intramuscularly with sporozoites, suspended in saline solution, obtained from six species of mosquitoes. Ten of the sparrows used in the first three experiments died, most of them between the fifth and seventh post-inoculation day. Their internal organs showed heavy infections with exoerythrocytic bodies. The response of these sparrows was apparently like the Pattern C infections reported by Haas *et al.* (1948). These workers infected chicks with sporozoites of *P. gallinaceum* from mosquitoes which had fed on chicks infected by blood inoculation. These chicks died between 9 and 15 days after inoculation; many exoerythrocytic bodies were found in their brains, but very few or no parasites were in the red cells. Sparrows used in the present study died in a shorter time; E.E. forms were found in the spleen and liver, but few or none in the brain. In many sparrows with heavy E.E. infections in the internal organs, E.E. bodies were also present in the peripheral blood.

The 11 canaries injected with sporozoites in these experiments all remained nega-

tive. This result is comparable to those of Redmond (1944) and of Hunninen *et al.* (1950). Redmond failed to infect pigeons by inoculation with sporozoites of *P. relictum* from mosquitoes fed on canaries, which had been infected by blood transfer. Similar results were obtained by Hunninen *et al.*, using a sparrow-mosquito-canary sequence.

The results of these experiments, reported on the basis of exoerythrocytic response are shown in table 4. Of the 53 sparrows inoculated 35 showed a very heavy, a heavy, or a moderate degree of infection with E.E. bodies; 15 had a few E.E. bodies, and only three (about five per cent) were negative. As previously stated, all canaries injected remained negative.

The 53 sparrows used in these experiments were wild-caught; it was not known whether they had naturally acquired *P. relictum* infections, in which E.E. forms might be present. It was decided, therefore, to examine 47 wild-caught sparrows as controls. Table 5 shows the result of the 47 sparrows examined, 32 (66 per cent) were negative for E.E. forms; 14 had a few or very few E.E. forms, and only one had a moderate number.

Applying the chi square test, it was found that the difference in infection rates between the inoculated and the control sparrows probably was not due to chance (P = less than 0.001), and that the higher rate of infection in the inoculated birds was the result of such inoculation. The quantitative distribution of the exoerythrocytic forms in both groups supports this conclusion.

DISCUSSION

The results of the experiments made to determine whether anopheline mosquitoes could be infected with *P. relictum*, which is usually transmitted by culicines, extend the earlier findings of Hunninen *et al.* (1950) that anophelines are highly susceptible to this parasite, as evidenced by heavy midgut infections, complete development of the sexual cycle, and the infectiveness of the sporozoites.

A. albimanus was the most susceptible of the anophelines, the infection rate approaching that of the control *Culex pipiens*. In decreasing order of susceptibility were *A. freeborni*, *A. quadrimaculatus* and *A. crucians*.

It was again demonstrated that the sexual cycle developed more slowly in anophelines than in the *Culex* controls. Usually from 17 to 20 days was required, but sometimes it was completed in only 12 days. Various workers have shown that the sexual cycle of *P. relictum* in the normal culicine host is accomplished in 7 to 10 days.

A larger number of oocysts developed in all of the species of mosquitoes used when they fed on sparrows infected with the sparrow strain of *P. relictum* than when they fed on sparrows infected with the starling strain of *P. relictum*.

Inoculation of sporozoites into 53 English sparrows produced infections, usually heavy, in all but three. The exoerythrocytic infections were so massive that more than half of the sparrows died between the fourth and tenth day after inoculation. Large numbers of E.E. bodies were found in the spleen and liver, but few in the brain. Parallel inoculations of sporozoites into 11 canaries failed to cause infection; neither exoerythrocytic nor erythrocytic forms developed.

The results reported here with *P. relictum* emphasize the desirability of studying host and parasite relationships among other species of avian malarias. The suscepti-

bility of various anopheline species to avian malaria parasites, and their ability to transmit them, should be further investigated.

SUMMARY

1. A study was made of the susceptibility of four species of *Anopheles* to *Plasmodium relictum*.

2. *A. albimanus* proved highly susceptible, 80 per cent became infected, compared with 91.5 per cent of the *Culex pipiens* controls.

3. *A. crucians* was the least susceptible, 33 per cent developed the infection, compared with 93.5 per cent of the controls.

4. *A. quadrimaculatus* and *A. freeborni* showed a susceptibility intermediate between the other two species.

5. Complete development of the parasite occurred in all four anopheline species.

6. The rate of development was slower in the anophelines than in the normal culicine host. In two species sporozoites appeared in the salivary glands as early as the 12th day after feeding, but usually the interval was 17 to 20 days.

7. A larger number of oocysts was found in mosquitoes that fed on sparrows infected with the sparrow strain of *P. relictum* than in mosquitoes that fed on sparrows infected with the starling strain of *P. relictum*.

8. Sporozoites from all five species of anopheline and culicine mosquitoes, when injected into sparrows, produced infections. Similar inoculations failed to produce infections in canaries.

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RESUMEN

1. Se estudió la susceptibilidad a *Plasmodium relictum* de cuatro especies de *Anófeles*.

2. *A. albimanus* demostró ser muy susceptible, el 80 por ciento desarrolló la infección en comparación con 91.5 por ciento de *Culex pipiens* que actuó como control.

3. *A. crucians* resultó ser el menos susceptible, el 33 por ciento desarrolló la infección en comparación con el 93.5 por ciento de los controles.

4. *A. quadrimaculatus* y *A. freeborni* demonstraron una susceptibilidad intermedia entre las otras dos especies.

5. El parásito se desarrolló completamente en todas las cuatro especies anofelinas.

6. El desarrolló fué más lento en los anofelinos que en el huésped *Culex* normal.

Los esporozoítos aparecieron en las glándulas salívales en dos especies tan temprano como el duodécimo día después de la alimentación pero el término común fué de 17 a 20 días.

7. Se encontró un número mayor de oocitos en los mosquitos alimentados en gorriones que habían sido infectados con la cepa gorrión de *P. relictum* que en los alimentados en gorriones infectados con la cepa estornino de *P. relictum*.

8. Se produjeron infecciones mediante la inoculación de gorriones con esporozoítos obtenidos en la cinco especies de mosquitos pertenecientes a los géneros *Anófeles* y *Culex*. Inoculaciones similares en canarios no produjeron infecciones.

THE IDENTIFICATION OF THE EARLY LARVAL INSTARS OF THREE COMMON ANOPHELINES OF SOUTHERN GEORGIA, U.S.A.[1]

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The anopheline species most common in Baker County, Georgia, are *Anopheles quadrimaculatus* Say, *A. punctipennis* (Say), and *A. crucians* Wiedemann. Less common are *A. perplexens* Ludlow[2], *A. georgianus* King, and *A. barberi* Coquillett, the latter breeding in tree-holes.

In connection with routine observations on the fluctuations in numbers of the common species of *Anopheles* found in selected localities in Baker County, Georgia, means of identifying all four larval instars were required.

Descriptions and keys to the identification of fourth-instar larvae of the anophelines of the Southeastern States are numerous (Root, 1924; Russell, 1925; Bradley, 1936; King, Bradley, and McNeel, 1939; King and Bradley, 1941; Ross and Roberts, 1943; Carpenter *et al.*, 1946; Ross, 1947). Hurlbut (1941) discussed the first instar larvae of the more common southeastern species, and Dodge[3] has discovered diagnostic characters for the first instar larvae of these and various other species. Second instar larvae appear to have been almost entirely neglected; Hurlbut (1938) included the chaetotaxy of second instar larvae of *Anopheles walkeri* Theobald in his paper on the four larval instars of this species. No keys are available for identifying anopheline larvae in the third instar, but characters of fourth instar larvae aid to some extent in recognizing third instars.

This study was made to provide means of identifying all the instars of the three most common anophelines of Baker County.

MATERIALS AND METHODS

Wild-caught females of each of the three common species were identified and isolated in lantern-chimney cages over bowls of water. Eggs deposited by these females were kept in the isolation bowls until the larvae emerged. The larvae from each egg-batch, the progeny of a single female, were reared to the fourth instar. A few larvae in each instar from a number of such isolations were preserved for study.

Most of the larvae were killed in hot water or in 70 per cent alcohol, and preserved

[1] I am indebted to Dr. R. Edward Bellamy for assistance and guidance, and to Gory J. Love and Robert P. Repass for rearing and preserving much of the material.

[2] The validity of *Anopheles perplexens* Ludlow was recently established by Bellamy (in press). He found that as fourth instar larvae, pupae, or adults, *perplexens* and *punctipennis* were almost indistinguishable. It should be recognized that "*punctipennis*" as used in this paper refers actually to the "*punctipennis* complex" and includes *perplexens*. Reliable morphological characters for distinguishing *perplexens* and *punctipennis* as first, second, or third instar specimens were not discovered.

[3] H. R. Dodge has a key for the determination of first instar specimens of a number of North American and Caribbean species of Anopheles. This unpublished key was made available to me by Dr. Dodge.

in the latter, but some were processed and mounted on slides immediately. The larvae were placed in 10 per cent KOH for several hours, then washed in water, placed on slides and run through a series of three solutions consisting of various proportions of glacial acetic acid and oil of cloves, then mounted in euparal. The steps of the process are as follows:

10 per cent KOH	—several hours
Water	—10 to 15 minutes
Solution	I—10 minutes (glacial acetic acid).
	II—10 minutes (a mixture of one part glacial acetic acid and one part oil of cloves).
	III—Minimum of 10 minutes; specimen may remain in this solution indefinitely (a mixture of one part glacial acetic acid and two parts oil of cloves).
Mount in euparal	

The acetic acid acts as an efficient dehydrating agent, and the gradual change to oil of cloves restores the normal shape of the specimen and helps in its clearing. The specimen may be handled after a minimum of ten minutes in solution III. Some specimens were mounted in Berlese's medium directly from water. Methods of preparing specimens for examination varied somewhat for each instar; these methods are discussed elsewhere.

FIRST INSTAR LARVAE

To test the reliability of the first instar characters given by Hurlbut (1941), 50 to 100 first instar larvae of each species, the progeny of at least five females of the there species, were mounted and examined. All these larvae without exception had the characters given in Hurlbut's key. Thus the problem of identifying first instar larvae of the three species was solved.

One aim of this study was to simplify the technique of identifying large numbers of larvae collected in natural breeding-places. Hurlbut (1941) recommended that first instar larvae be examined with a compound microscope using a 4 mm. objective. It was found that most first instar larvae of *A. punctipennis* could be identified positively, using a binocular dissecting microscope with about 54× magnification.

The following procedures were found to be the most satisfactory for identifying larvae preserved in alcohol. Hurlbut's characters for first instar larvae were used. The nomenclature of the larval hairs is that of Martini as modified by Root (1924).

A few first instar larvae are placed in each depression of a white porcelain color reaction plate. Examine the subantennal hairs (hair 12, fig. 1, A) with a dissecting microscope, using 54× magnification. Specimens having the subantennal hairs with lateral branches on one side only (fig. 1, A) are *A. punctipennis*, and are separated from the other specimens. Larvae in which the subantennal hairs are not clearly visible, or with these hairs obviously with lateral branches on both sides of the shaft (fig. 1, B and C) are mounted and examined under a compound microscope, using a 4 mm. objective. In our region this latter group of larvae includes a few specimens of *A. punctipennis* in which the subantennal hairs are obscured, and all specimens of *A. quadrimaculatus* and *A. crucians*. Identification of *punctipennis* is facilitated by

flattening the heads of the larvae during mounting, to bring the subantennal hair into view. The larvae must be mounted with the dorsum uppermost, as the larvae of *crucians* and *quadrimaculatus* are separated by differences in the dorsal hairs of abdominal segments VI and VII (fig. 1, B and C).

The characters used by Hurlbut (1941) to distinguish first instar larvae are: Larvae with hair 1 of abdominal segment VII lanceolate, and with hair 5 of abdominal segments VI and VII bifurcate near the base (fig. 1, C) are *quadrimaculatus*. Larvae with hair 1 of segment VII simple, and with hair 5 of segments VI and VII unbranched (fig. 1, B) are *crucians*. Living larvae are sometimes more easily identified

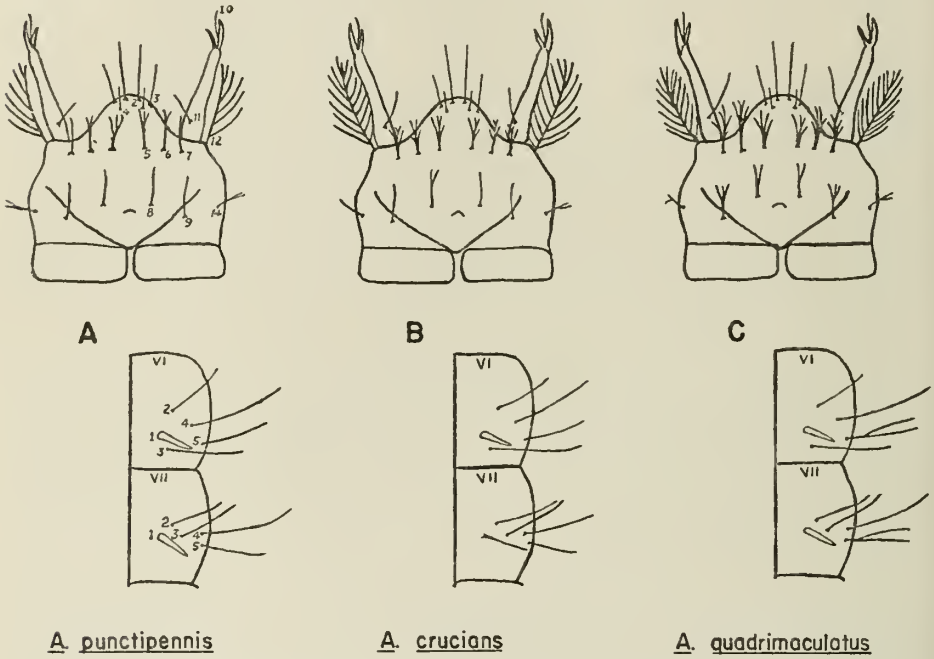

FIG. 1. Head and right half of abdominal segments VI and VII of first-instar larvae of *A. punctipennis*, *A. crucians*, and *A. quadrimaculatus*.

than preserved specimens, because they tend to rest dorsum uppermost. They are examined in a small amount of water under a cover-slip. The pressure of the cover-slip immobilizes the larvae, so that they may be examined under a compound microscope.

SECOND INSTAR LARVAE

Keys have not previously been made for the identification of second instar larvae of *A. punctipennis*, *quadrimaculatus*, and *crucians*. Slidemounts and a compound microscope are necessary to identify these instars. The most useful diagnostic characters found are given in the following key.

1. Ventral prothoracic hair No. 14 small, with not more than 4 branches; antennal hair arising at, or within, basal fourth of antenna (Fig. 2, B)..... *punctipennis*

Ventral prothoracic hair No. 14 larger, with 6 or more branches; antennal hair (No. 11, Fig. 2, A) arising beyond basal fourth of antenna (Fig. 2, A and C)..... 2

2. Inner clypeal hairs (No. 2, Fig. 2, A) widely separated at base, the tubercles from which they arise separated by a distance greater than the diameter of one of these tubercles, often by two or three times the tubercle diameter; outer clypeal hairs (No. 3, Fig. 2, A) thinly branched, both hairs together averaging less than ten branches; antennal hair arising at, or beyond, basal third of antenna (Fig. 2, A)..... *quadrimaculatus.*
Inner clypeal hairs closely approximated at base, the basal tubercles from which they arise separated by a distance no greater than the diameter of one of these tubercles; outer clypeal hairs more branched, both hairs together averaging at least 10 branches; antennal hair usually arising within basal third of antenna (Fig. 2, C)..... *crucians*

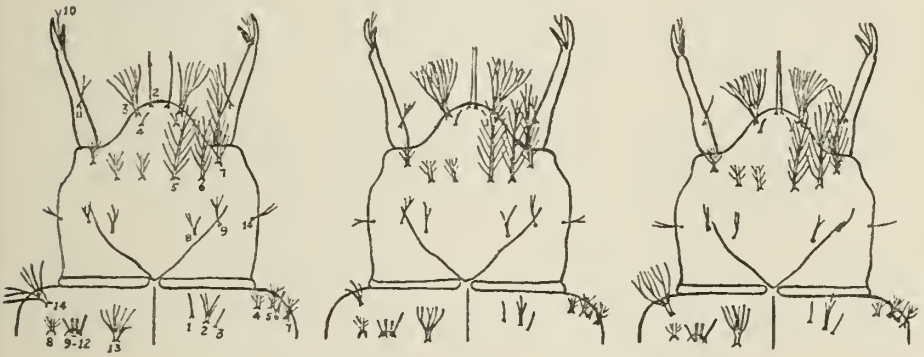

FIG. 2. Head and prothorax of second instar larvae of *A. quadrimaculatus*, *A. punctipennis*, and *A. crucians*. The head of each species is shown in dorsal aspect; the right half of each prothorax is dorsal, the left half ventral. Head hairs: 2, inner clypeal; 3, outer clypeal; 4, post clypeal; 5–7, frontal; 8–9, occipital; 10, terminal antennal; 11, antennal; 14, orbital.

THIRD INSTAR LARVAE

Comparison of the characters used to identify fourth instar larvae of *punctipennis*, *quadrimaculatus*, and *crucians* was made with similar characters in third instar larvae of these species. Only about 80 per cent of the third instars were correctly identified, using these characters. Therefore additional distinguishing characters for third instar larvae were sought. Some characters useful in identifying second instars were found to be applicable to third instars. The following key to third instar larvae of *quadrimaculatus*, *punctipennis*, and *crucians* uses a combination of characters found in second and fourth instars.

1. Inner clypeal hairs widely separated at base, the tubercles from which they arise separated by a distance greater than the diameter of one of these tubercles, often by a distance equal to, or exceeding, twice the tubercle diameter; inner occipital hairs with five or more branches; outer occipital hairs with four or more, usually five or more branches; antennal hair arising at, or beyond, basal third of antenna. (Hair 2 of abdominal segments IV and V single.) (Fig. 3, A)..... *quadrimaculatus.*
Inner clypeal hairs closely approximated at base, the tubercles from which they arise separated by a distance no greater than the diameter of one of these tubercles; occipital hairs not more

than four-branched, usually two- or three-branched; antennal hair usually arising within the basal third of the antenna. (Hair 2 of abdominal segments IV and V normally double or triple, rarely single.) (Fig. 3, B and C)..... 2

2. Hair 0 of abdominal segments IV and V easily distinguishable; ventral prothoracic hair 14 large and heavily branched (Fig. 3, C).....*crucians*.
Hair 0 of abdominal segments IV and V absent or rudimentary; ventral prothoracic hair 14 small and thinly branched (Fig. 3, B).....*punctipennis*

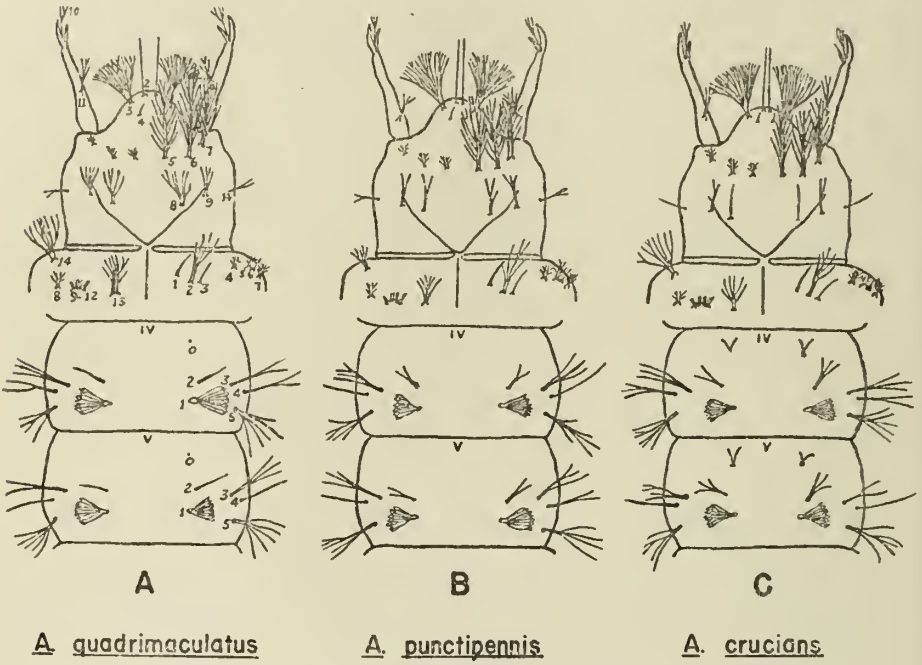

FIG. 3. Head, prothorax, and abdominal segments IV and V of third instar larvae of *A. quadrimaculatus*, *A. punctipennis*, and *A. crucians*. The head and abdominal segments four and five are shown in dorsal aspect. The right half of each prothorax shows the dorsal hairs, the left half ventral. Head hairs: 2, inner clypeal; 3, outer clypeal; 4, post clypeal; 5–7, frontal; 8, inner occipital; 9, outer occipital; 10, terminal antennai; 11, antennal; 14, orbital.

DISCUSSION

The use of a dissecting microscope permits the separation of most first-instar specimens of *A. punctipennis* from other first-instar larvae. This is possible because the conspicuous unilaterally branched subantennal hair is diagnostic for *punctipennis*. First-instar specimens of *A. quadrimaculatus* and *A. crucians* must be mounted for examination with a compound microscope if they are to be identified, and some specimens of *A. punctipennis* with partly obscured subantennal hairs must also be mounted. Berlese's medium was extremely useful, as specimens could be placed in it directly from water. A number of larvae may be mounted rapidly on a single slide by placing them dorsum uppermost in individual drops of the mounting medium. The larvae are easily transferred on the point of a dissecting needle.

Second-instar *A. punctipennis* larvae are easily distinguished from *A. quadrimacu-*

TABLE 1

Results of examination of second-instar characters

Character Examined																							
Head-hair 2 (inner clypeal hair)						Head-hair 3 (outer clypeal hair)						Head-hair 11 (antennal hair)						Ventral prothoracic hair 14					
Separated at base by twice diameter of tubercle		Separated at base by less than twice diameter of tubercle				Average of both hairs less than 10 branches, usually 7 or 8				Average of both hairs at least 10 branches, usually 12 or 13		Inserted at or beyond basal third of antenna		Inserted at or within basal fourth of antenna		Inserted beyond basal fourth of antenna		Large, not less than 6-branched		Small, not more than 4-branched		Large, not less than 6-branched	
*quadrimaculatus**		*punctipennis***		*crucians****		*quadrimaculatus*		*punctipennis*		*crucians*		*quadrimaculatus*		*punctipennis*		*crucians*		*quadrimaculatus*		*punctipennis*		*crucians*	
Number examiued	Number Agreeing	Number Examined	Number Agreeing	Number Examined	Number Agreeing	Number Examined	Number Agreeing	Number Examined	Number Agreeing	Number Examined	Number Agreeing	Number Examined	Number Agreeing	Number Examined	Number Agreeing	Number Examined	Number Agreeing	Number Examined	Number Agreeing	Number Examined	Number Agreeing	Number Examined	Number Agreeing
54	49	92	84	49	49	45	45	Extremely variable 3–13 branches		42	41	61	60	95	94	51	48	75	75	49	49	40	40

* Origin of larvae: Eggs from six wild-caught females, and a few from N. I. H. stock colony.

** Origin of larvae: Eggs from eight wild-caught females.

*** Origin of larvae: Eggs from four wild-caught females.

TABLE 2

Results of examination of third-instar characters

	A. quadrimaculatus Offspring of 10 wild females represented	*A. punctipennis* Offspring of 8 wild females represented	*A. crucians* Offspring of 8 wild females represented
Head hair 2 (inner clypeal hair)	65 specimens examined. In 63 specimens, separated at the tubercle base by at least twice the diameter of one tubercle. In 2 specimens, separated at the tubercle base by at least the width of one tubercle.	Usually closely approximated as in. *A. crucians.*	48 specimens examined. In all specimens, separated at the tubercle base by no more than the diameter of one tubercle.
Head hairs 8 and 9 (occipital hairs)	60 specimens examined. Hair 8. None less than five branched. Hair 9. In 58 specimens, at least five branched; in 2 specimens, four branched.	48 specimens examined. Hair 8. None more than four branched. Hair 9. None more than four branched.	45 specimens examined. Hair 8. None more than three branched. Hair 9. In 43 specimens, less than four branched; in 2 specimens, four branched.
Head hear 11 (antennal hair)	65 specimens examined. In all specimens, arises at or beyond the basal third of the antenna.	51 specimens examined. In all specimens, arises well within the basal third of the antenna.	46 specimens examined. In 30 specimens, arises within the basal third of the antenna. In 16 specimens, arises slightly beyond the basal third of the antenna.
Prothoracic hair 14	61 specimens examined. Large in all specimens, not less than nine branched.	48 specimens examined. Small in all specimens, not more than six branched.	45 specimens examined. Large in all specimens, not less than 10 branched.
Hair 2, abdominal segments IV and V.	65 specimens examined. Single in all positions of each specimen.	53 specimens examined. Usually double. Occasionally single. Rarely triple.	47 specimens examined. Usually triple. Occasionally double. Rarely single or quadruple.
Hair 0, abdominal segments IV and V.	65 specimens examined. Absent or rudimentary in all.	53 specimens examined. Absent or rudimentary in all.	47 specimens examined. Usually present as shown in figure 3, C. Occasionally not visible at low magnification.

latus and *A. crucians* by the position of the antennal hair and by the size and number of branches of the ventral prothoracic hair 14. In combination, these characters are extremely reliable. *A. quadrimaculatus* and *A. crucians* larvae may then be separated by the characters of the inner and outer clypeal hairs, and the site of insertion of the antennal hair. The data presented in Table 1 illustrates the relative reliability of the various characters used.

Second-instar larvae must be viewed with a compound microscope for identification. The larvae may be mounted in the same manner as first-instar specimens. It is helpful to stretch the head and thorax apart so that the ventral prothoracic hair 14 is not obscured by the head-capsule.

When applied to third-instar larvae, fourth-instar characters are of limited application. The most frequently encountered difficulties were due to variations in the branching of hair 2 on abdominal segments IV and V in *punctipennis* and *crucians*, and to the frequent difficulty in seeing hair 0 on the same segments of *crucians* larvae. Hair 2 on abdominal segments IV and V of *punctipennis* and *crucians* larvae may be single, double, or triple. The character of the ventral prothoracic hair 14 and of hair 0 on abdominal segments IV and V is helpful in distinguishing *punctipennis* from *crucians*. The obvious presence of hair 0 on abdominal segments IV and V establishes the identity of a specimen as *crucians*, but in some larvae of this species hair 0 is very difficult to see. When visible it is almost always as shown in Fig. 3, C. *A. quadrimaculatus* is easily distinguished by the characters of the inner clypeal, occipital and antennal hairs. Hair 2 on abdominal segments IV and V is also consistently single in this species. The relative reliability of the third-instar characters is indicated by the data in Table 2.

The key to third-instar larvae is for use with a compound microscope; however, specimens in good condition may be identified with a dissecting microscope with 54× magnification. If mounted, third-instar larvae should first be cleared in 10 per cent KOH and the head and thorax should be stretched apart during the mounting process. Again, Berlese's medium was found to be very satisfactory.

With the foregoing keys and procedures for identification of first-, second-, and third-instar larvae, methods are available for distinguishing *A. quadrimaculatus*, *A. punctipennis*, and *A. crucians* in any stadium, since diagnostic keys applicable to fourth-instar specimens of these species are well known, and the pupae are treated in papers by Darsie (1949) and Penn (1949).

It is hoped that the findings presented here may be useful to workers in other areas. It must be kept in mind, however, that the material used in this study was obtained from a restricted locality, and differences may be found in larvae from other regions because of geographical variations. It is hoped that future studies may broaden the scope of this work to include representative specimens from other areas and possibly other species.

SUMMARY

Methods for distinguishing *A. quadrimaculatus*, *A. punctipennis*, and *A. crucians* in all stadia were developed through a study of representative material from Baker County, Georgia. Keys for second- and third-instar larvae are provided. A practical

procedure for using Hurlbut's (1941) characters in the mass identification of first-instar larvae is offered. This paper makes it possible to identify *A. quadrimaculatus*, *A. punctipennis*, and *A. crucians* in any stadium, since keys to fourth-instar larvae and pupae are already available. Salient characters of first-, second-, and third-instar specimens are illustrated, and tables summarizing the occurrence of these characters in the representative material studied are given.

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RESUMEN

Se desarrollaron métodos para distinguir *Anopheles quadrimaculatus*, *A. punctipennis* y *A. crucians* en todos estados a través de un estudio de material representativo procedente de "Baker County, Georgia". Incluyéronse las claves para las fases larvarias 2 y 3. Se ha ofrecido un procedimiento práctico para el uso de los caracteres de Hurlbut (1941) en la identificación en masa de larvas en su primera fase evolutiva. Este artículo hace posible la identificación de *A. quadrimaculatus*, *A. punctipennis*, y *A. crucians* en cualquier estado de crecimiento ya que se puede hacer uso ahora de claves para larvas y pupas en su cuarta fase evolutiva. Se han ilustrado los caracteres sobresalientes de especímenes en sus fases evolutivas 1, 2 y 3 y el establecimiento de estos caracteres en el material representativo estudiado aparece compendiado en las tablas incluídas.

DISTRIBUTION AND CONTROL OF MOSQUITOES IN RICE FIELDS IN STANISLAUS COUNTY, CALIFORNIA

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The first commercial rice crop in California was grown in 1912 in the Sacramento Valley near Biggs. Jones (1940) reports that the high acre yields and large profits from this crop of 1,400 acres received wide publicity, which stimulated interest and resulted in the rapid expansion of the California rice industry. In 1920, 162,000 acres were sown; while in 1947, the crop was grown on approximately 250,000 acres.

Rice is grown in California principally in the Sacramento and San Joaquin Valleys. Continuous flooding of the land to a depth of four to seven inches, throughout the growing season of 90 to 140 days, is necessary for the maturation of the rice. The rice fields, extensive bodies of shallow, warm water with relatively dense vegetation, are prolific sources of mosquitoes. Soon after the fields are flooded in the spring, the "checks" (small plots or sections of a rice field bounded by low levees) produce large numbers of *Aedes* mosquitoes, followed by *Culex* and *Anopheles* in the summer. Aside from the rice fields proper, the problem is further complicated by mosquito-breeding in drainage ditches, roadside pools, and seepage areas. Thus, as pointed out by Freeborn (1917), the cultivation of rice through the years has increased the number of mosquitoes in proportion to the phenomenal growth of this type of agriculture. Because of the extensive areas involved, the control of rice field mosquitoes in California is the most difficult problem yet encountered in mosquito abatement.

Anopheles maculipennis freeborni Aitken is the principal vector of malaria in California. Recent studies have indicated that the principal vector of St. Louis encephalitis and of Western equine encephalomyelitis in California is *Culex tarsalis* Coquillett (Hammon and Reeves, 1943).

The St. Louis and Western encephalitides have been known to exist in California for a considerable time, and have been present in epidemic form among human beings in several areas, including the San Joaquin Valley; Western equine virus has been present among horses in many parts of the State (Anon., 1945). The two species of mosquitoes just mentioned were found in significant numbers in all of the rice fields under observation during this study.

Since it is difficult to inspect rice fields for mosquito larvae except by dipping from the levees, the relative productivity of the central portions of the "checks" has not heretofore been adequately measured. Wading across rice fields is not only difficult, and likely to affect the number of larvae to be found at the surface, but it is destructive to the rice plants. By toppling stems into the water, conditions are made favorable for mosquito production. Because of these conditions, it has been difficult to obtain satisfactory evaluations of control measures, particularly of the applications of DDT and DDD from the air.

In the present study, three "checks" were selected in three different rice fields. One "check" was allowed to remain untreated throughout the season as a control. The second was sprayed with an aqueous emulsion of DDT, and the third was sprayed with an aqueous emulsion of DDD (dichlorodiphenyl dichloroethane), an analogue of DDT. Applications were made from a Piper Cub plane used by the local mosquito abatement district in aerial spray operations. A dosage of approximately 0.3 pound of DDT and of DDD per acre was used.

A review of the literature showed that several investigators had made observations on the distribution of mosquito larvae in rice fields. Geiger and Purdy (1919) in observations near Lonoke, Arkansas, reported that larvae found along levees in one set of plots comprised 64 per cent of the total, while in another set the numbers of larvae along the levees and in the plot interiors were about equal. In the Nadia District of Bengal, Sur (1931) found that mosquito breeding takes place not only at the edges, but over the entire surface of rice fields. Robertson and Chang (1937) present an account of the relation between rice cultivation and the breeding of *Anopheles hyrcanus* var. *sinensis* Wiedemann, the local vector of malaria in the Kaochiae district of Shanghai, China, based on observations of irrigation ditches, stagnant pools, and flooded rice fields. They report that the larvae were confined to the edges of ditches and pools, but tended to spread over the whole field where the water surface was covered with aquatic vegetation. Cambournac (1939) reports that larvae were found throughout the rice field, not merely at the edges. Differences in density could be correlated with the presence of *Lemna*, which limits the number of larvae and of filamentous algae, especially *Spirogyra*, which are food and protection for the larvae. In rice fields in California, Aitken (1945) found that although larvae are found mainly around the margins, they also may occur at considerable distances from the levees, and are not necessarily associated with algae. In the opinion of Horsfall (1946), the sampling of populations of larval mosquitoes in rice fields presents a unique problem because the fields provide uniform habitats of extensive areas. These fields are nearly flat, with contour levees retaining water to a depth of 4 to 8 inches. Horsfall found that larvae, when present, were usually distributed over the whole area.

METHODS

The rice fields selected for study were located in Stanislaus County, approximately five and a half miles northeast of the city of Modesto, California. The fields in this area were divided by levees into rectangular "checks" of various sizes, and were close to each other. Three rice fields were selected, the Krause, Petersen, and Frobose fields.

One "check" in each of the Krause and Petersen rice fields was selected because of: (a) convenient size, (b) ease of access, (c) production of a uniform and good growth of rice, as determined by the stubble of the previous crop, and information obtained from the owner, and (d) continuous flooding during the growing season. A third "check" in the Frobose field was selected because it contained numerous cattails, and it was deemed desirable to compare this "check" with other "checks" containing almost pure stands of rice.

To avoid disturbing the natural larval habitat, plank bridges extending north to

south from levee to levee were constructed in each of the three study "checks". As these bridges were installed after the fields had been plowed or disked, care was taken to confine all operations as closely as possible to the path of the bridge in order to prevent packing of the soil. After the piers of the bridges, consisting of two 4-foot 4 by 4's placed upright two feet apart at intervals of approximately 10 feet, had been placed, the trampled soil was carefully loosened with shovel and rake.

The 2 by 12-foot planks of the bridge floors were placed after each "check" had been seeded and flooded, without disturbing the interior of each study plot. After flooding, each bridge was approximately one foot six inches about the water surface. The Krause "check" had a flooded area of approximately 42,350 square feet, its

FIG. 1 Krause study check showing the planks of the bridge. Dips from the center were started at a distance from the platform and ended at least two feet from it in order to avoid dipping close to the platform. Later in the season the bridge was obscured by tall rice.

dimensions being 385 x 110 feet; the Petersen "check" contained about 10,480 square feet, being 131 x 80 feet; the Frobose "check" was L-shaped, 80 feet in width, with an area of approximately 47,588 square feet.

While making observations on larval abundance, the inspector proceeded from south to north on the bridge across each study "check", and made one dip at each station, designated at intervals of two feet on each side of the bridge. Thus, each 12-foot plank represented a 5-dip area on each side of the bridge, making a total of 10 dips for each plank length. The technique of dipping for larvae from a bridge is shown in Figure 1.

To compare the incidence of mosquito larvae in the middle of the "check" with that occurring along the levees, an equal number of dips was made in each station at each observation. The mid-field area of each study "check" was considered to be all

the area traversed by the bridge, minus the first and last planks at each end. Thus, in the Krause study "check", the first and last planks were used only as approaches to the center planks; all dips made near these two end planks were within the levee dipping area. Since the center of the bridge, excluding the two end planks, was 9 plank lengths, each observation involved making 45 dips on each side of the bridge, a total of 90 dips. Therefore, 90 dips were made at equally distributed stations around the perimeter or levees of the "check". In the Petersen and Frobose study "checks", respectively, the above procedure included six planks and involved 30 dips on each side of the bridge, a total of 60 dips. The same number of dips was made along the levees.

The dipping stations along the levee were marked with laths stuck upright in the study "check" in straight rows near the perimeter. These laths were numbered consecutively for each "check", after the bridge stations had been numbered, beginning at the relative position in each "check". The numbered levee stations were in turn subdivided into so-called A, B, and C sections of the stations. These sections of the stations were indicated by marking two laths, marked A and C on opposite sides of each, to indicate corresponding sections. The B section was unmarked, as it was the middle area between two laths, with the insides marked A and C.

White enameled dippers of one-pint capacity were used. The dippers were attached to 5-foot bamboo or steel poles. From early May to early June, dips were made by moving the dipper away from the bridge or levee. It proved difficult to dip in this direction in shallow water and in dense vegetation, so the method used later throughout this study was to move the dipper toward the bridge or levee. The dipper was placed in the water at a slight angle, four or five feet from the bridge, and drawn toward the observer through a distance of about three feet. Dipping close to the bridge was avoided. Dips were made every two feet along the bridge. The observers were able to space their dips quite accurately by measuring the distance with their feet as they proceeded along the bridge.

A portable stand equipped with a white enameled pan and two one-quart jars was used. The contents from each dip were poured into the pan, the larger vegetation and debris removed, and the water allowed to become still. The large larvae and pupae of the anophelines were counted and placed in one jar; the large larvae and pupae of the culicines were likewise counted and placed in the same jar. The smaller anopheline and culicine larvae were counted and placed in the other jar. The water in the pan was stirred several times, to dislodge any larvae hidden by the smaller floating particles. Any thus found were placed in the appropriate jar. Newly-hatched larvae were more easily detected in the pan than in the dipper.

After it was certain that all the data were recorded, the smaller larvae were poured back into the pan. As the observer walked along the station, the water in the pan was slowly poured back into the "check", care being taken that the contents were emptied along the whole station at least two feet from the bridge. The Krause study "check" was inspected three times each week (Monday–Wednesday–Friday); Petersen "check", twice a week (Tuesday and Thursday); and Frobose "check", twice a week (Monday and Friday).

The large larvae and pupae were taken to the laboratory for rearing.

Collections made from the bridge and levee stations were kept separate. Approximately 20 larvae and pupae were taken from the bridge stations (morning and afternoon collections) for rearing purposes, and the same number from the levee stations during almost all of the inspection periods. These collections were used mainly to rear adults for identification purposes, since as far as the species involved in this study are concerned, the identification of the adult stage is in general more reliable than that of the larval stage.

The method of dipping across both sides of the bridge was more or less constant, except for one deviation. Both slow and quick dips were used and alternated at each complete inspection. Along the levees, however, not only was the type of dip alternated as each complete inspection was made from the bridge, but the A, B, and C stations also were rotated. Thus, for example, in a "check", the "A" stations of the levee and all bridge stations were inspected with slow dips on one day; at the next observation, the "B" stations and all bridge stations were inspected with quick dips; and at the next observation, "C" stations of the levee and all bridge stations with slow dips.

To avoid the effect of shadows while dipping along the bridge, the even-numbered stations on the east side of the bridge were dipped during the morning, and the odd-numbered stations on the west side during the afternoon. Dips along the levees were begun following the morning inspection along the east side of the bridge. The procedure used was to follow the consecutive numbers starting at the station nearest the bridge on the north side and proceed counterclockwise around the levee. The entire levee was dipped between the hours of 10 A.M. and 3 P.M., the total time depending on the incidence of larvae. Thus, all dips from the bridge and all dips from the levee were made while facing the sun, and shadows were eliminated as a complicating factor.

OBSERVATIONS

The dominant emergent plants in the Krause "check" were two grasses, a domestic rice of the "Late Wataribune" variety, and the grass commonly referred to as "watergrass" or barnyard grass, *Echinochloa crusgalli*, long known as a serious pest in the Sacramento Valley rice fields. In the Petersen "check", in addition to these, a wire grass, *Eleocharis palustris*, was abundant around the levees, being particularly dense at the corners. In the Frobose "check", which contained a poor stand of the "Late Wataribune" variety of rice and watergrass, there was also a very dense well-distributed growth of a cattail, *Typha latifolia*.

On September 17, the mean height of the vegetation in the Krause "check" was found to be 39.37 inches along the center, and 39.59 inches along the levees. Along the center of the Petersen "check" the mean height was 37.73 inches, and along the levees, 34.56 inches. Measurements along the center only of Frobose "check" showed the mean height to be 41.38 inches. Measurements on the density of the vegetation in the Krause and Petersen "checks", on the whole showed no apparent differences.

Water flowed in and out of all "checks" practically every day. In the Krause "check" the water fluctuated in depth between 7.94 inches and 10.95 inches during most of the season. In the Petersen "checks", the water depth fluctuated between

9.38 and 11.58 inches, and the water depth fluctuated between 7.06 inches and 9.32 inches in the Frobose "check".

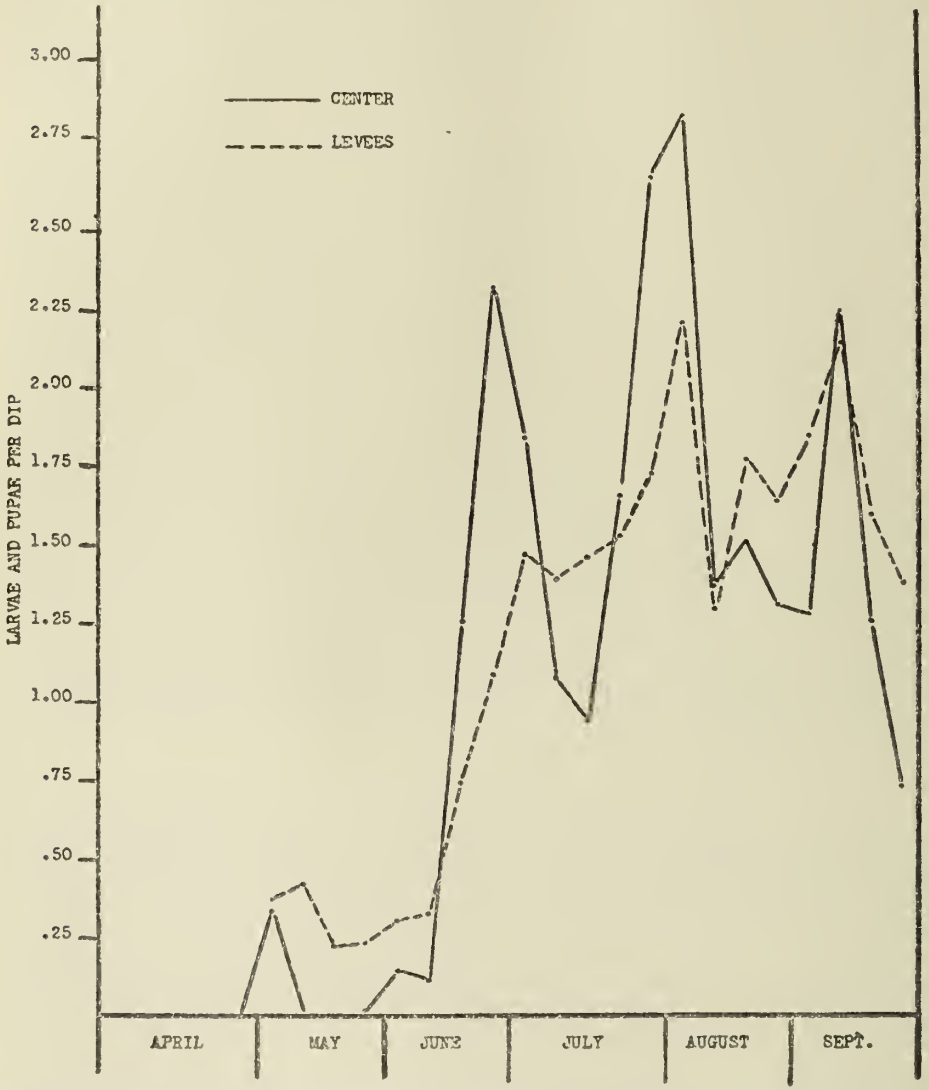

FIG. 2 Mean total number of mosquitoes from center and levees of Krause "check" (unsprayed) April–September, 1947. (Weekly mean number of larvae and pupae per dip).

After September 1, 1947, which was the official end of the irrigation season, the water in the Krause and Petersen fields gradually lowered at the rate of a quarter of an inch per day until the "checks" became dry, while the Frobose "check" soon was completely drained.

Observations during the early part of April, shortly after the "checks" had been

flooded, revealed heavy densities of *Aedes* larvae. Adults of *Aedes dorsalis* (Meigen), *Aedes nigromaculis* (Ludlow), and *Aedes vexans* (Meigen) were reared. This early abundance of larvae lasted for only two weeks. For example, the Krause "check" was completely flooded April 14–15, 1947, and on April 23 the "checks" were heavily infested with *Aedes* larvae. Examination of the "checks" five days later revealed no

FIG. 3 Anopheles and Culex from entire Krause "check" (unsprayed) April–September, 1947. (Weekly mean number of larvae and pupae per dip).

mosquito larvae or pupae. The larvae were principally confined to the edges, particularly on very windy days. However, when the water was calm, there was a tendency for some of the larvae to occur along midfield. There was no vegetation in the "checks" at this time. *Aedes* larvae were not found in the various "checks" during the remainder of the season.

In the Krause "check", a total of 9,054 dips was taken, of which 4,224 dips were taken from the levees and 4,830 dips from the center. A total of 4,081 dips was taken

from the Petersen "check", 1931 dips from the levees, and 2,150 dips from the center. In the Frobose "check", a total of 4,329 dips was taken, of which 2,019 were from the levees and 2,310 from the center. The discrepancy in the total number of dips from the levees, as compared with the dips from the center, is due to the fact that lowering of the water in the "checks" dried a few of the levee stations.

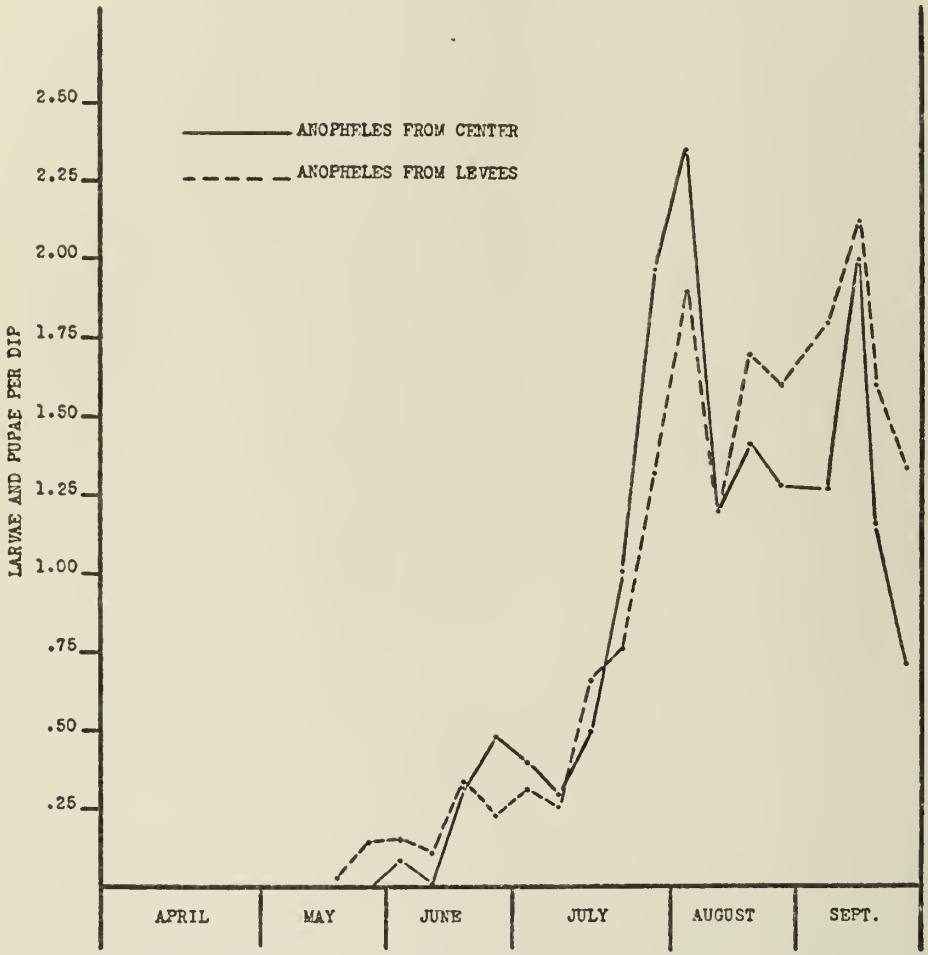

FIG. 4 Anopheles from center and levees of Krause "check" (unsprayed) April–September, 1947. (Weekly mean number of larvae and pupae per dip).

The criterion of comparison in this study is based on the numbers of larvae and pupae found in all dips taken from the bridge or platform (minus the first and last plank of each bridge), with the numbers found in an equal number of dips taken along the edges or levees of each "check". Thus, the contents of 90 dips taken along both sides of the platform are compared with those from 90 dips taken at regular intervals along the levees of the Krause "check". In each of the other "checks", 60 dips from the bridge are compared with an equal number from the levees.

KRAUSE "CHECK" (UNSPRAYED CONTROL PLOT)

A comparison of the mean total larvae and pupae throughout the season from the center and levees of the Krause "check" is shown in Figure 2, while Figure 3 shows the sequence of prevalence of *Anopheles* and *Culex* larvae and pupae in the "check". Immediately after spring flooding, *Aedes* larvae appear, then quickly disappear, to

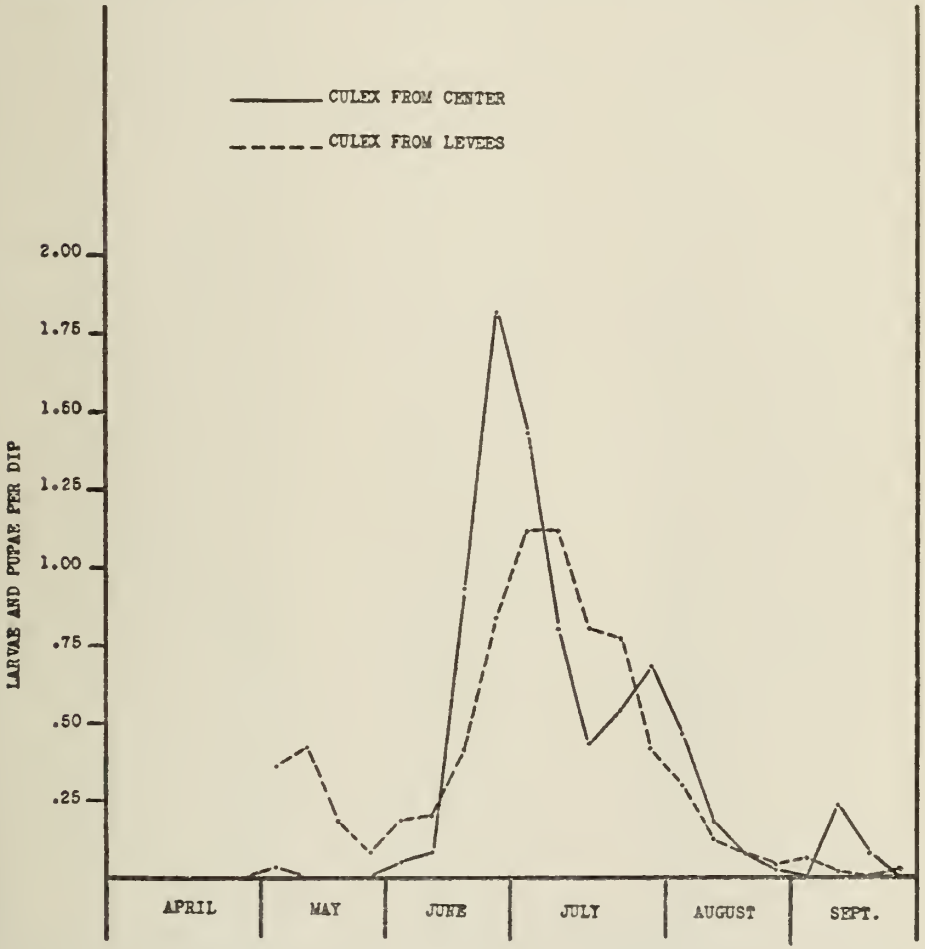

FIG. 5 Culex from center and levees of Krause "check" (unsprayed) April–September, 1947. (Weekly mean number of larvae and pupae per dip).

be followed in early summer by *Culex* larvae. In late summer, *Anopheles* larvae dominated the "check". The weekly mean number of *Anopheles* larvae and pupae from the center and levees is shown in Figure 4. *C. tarsalis* and *A. freeborni* were reared from collections made in the center and along the levees of the "check"; these species were the dominant larvae found during most of the season. However, adults of *Culex erythrothorax* Dyar were reared in very small numbers from larvae and pupae collected from the edges of the "check" during the first week of September. None were

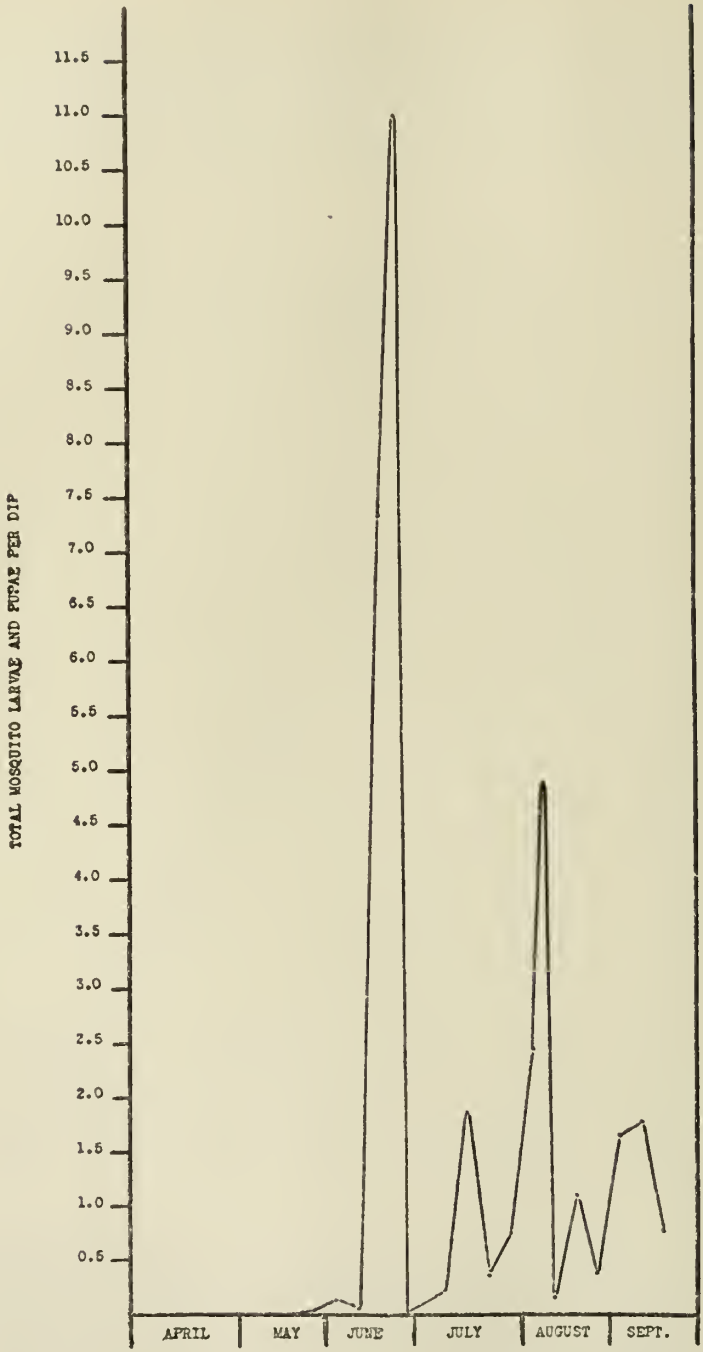

FIG. 6 Mean total number of mosquitoes from center of Petersen "check" (Spray "check" no. 1) April–September, 1947. (Weekly mean number of larvae and pupae per dip). "Check" sprayed June 25 and August 13, 1947.

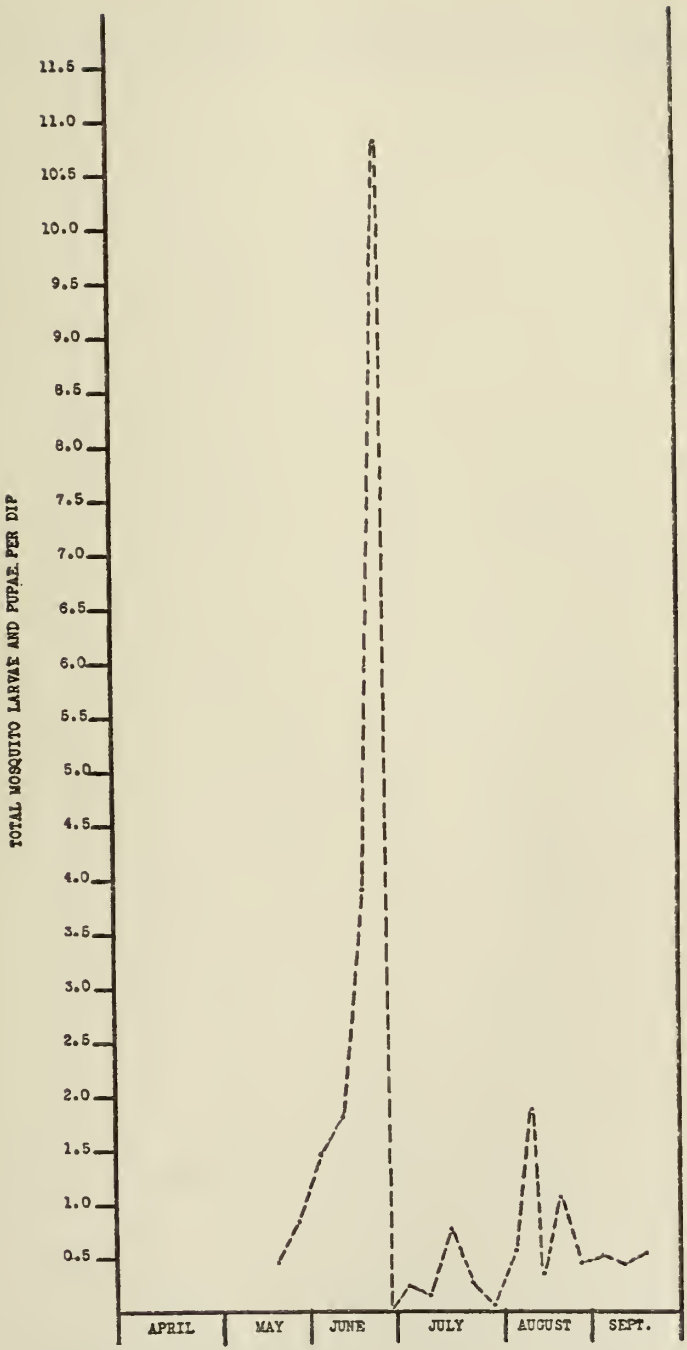

FIG. 7 Mean total number of mosquitoes from levees of Petersen "check" (Spray "check" no. 1). April–September, 1947. (Weekly mean number of larvae and pupae per dip). "Check" sprayed June 25 and August 13, 1947.

reared from collections made in the center. The weekly mean number of *Culex* larvae and pupae collected from the center and from the levees is shown in Figure 5.

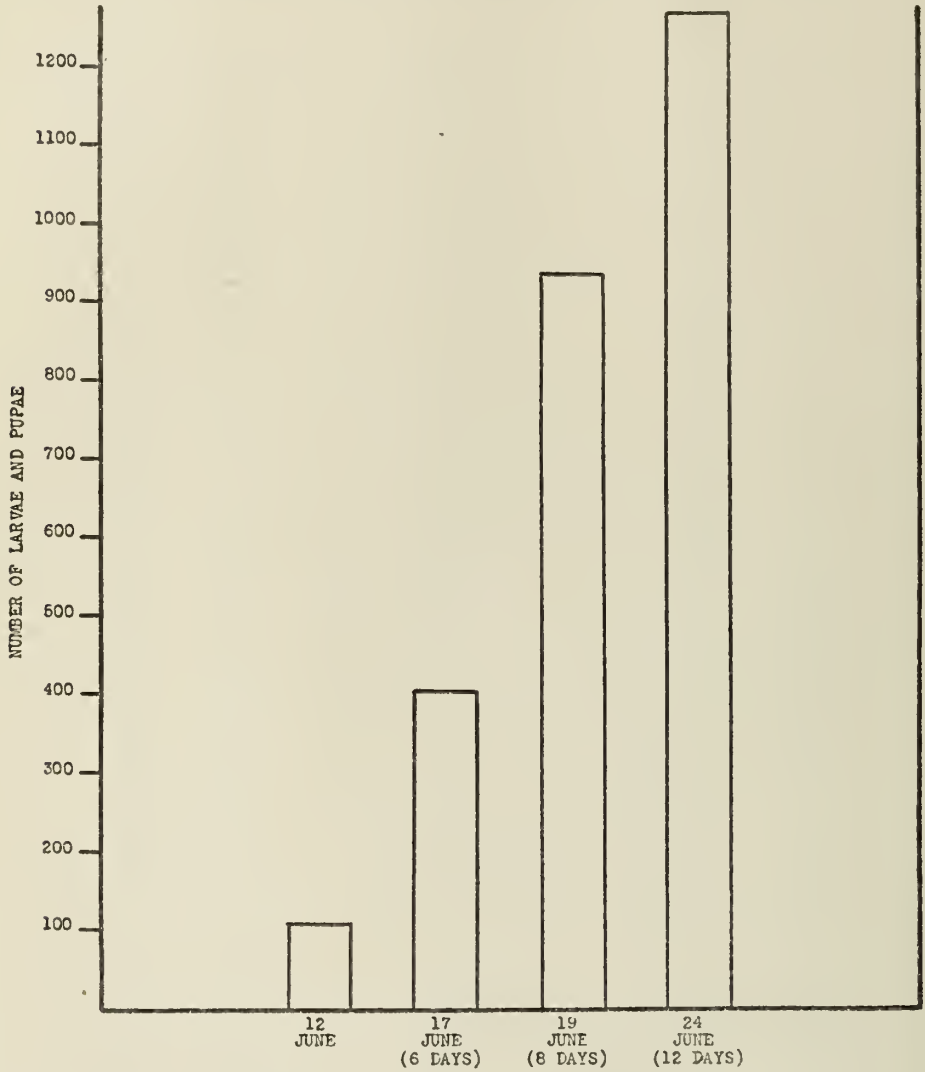

FIG. 8 *Culex tarsalis* larvae and pupae from Petersen "check" (Spray check no. 1) June 12–24, 1947. (Total larvae and pupae per 120 dips). "Check" sprayed June 25 and August 13, 1947.

PETERSEN "CHECK" (SPRAYED WITH DDT EMULSION)

The mean total of larvae and pupae collected from the center and levees of the Petersen "check" is shown in Figures 6 and 7. This "check" was sprayed twice during the season with aqueous emulsions of DDT (June 25 and August 13, 1947).

Only two species, *C. tarsalis* and *A. freeborni*, were reared from the larval and pupal collections taken during the season from the center and levees of the "check".

This "check" produced very large numbers of *C. tarsalis*. The rapid increase in numbers of *C. tarsalis* larvae and pupae, during a period of 12 days from June 12 through June 24, 1947, is graphically presented in Figure 8.

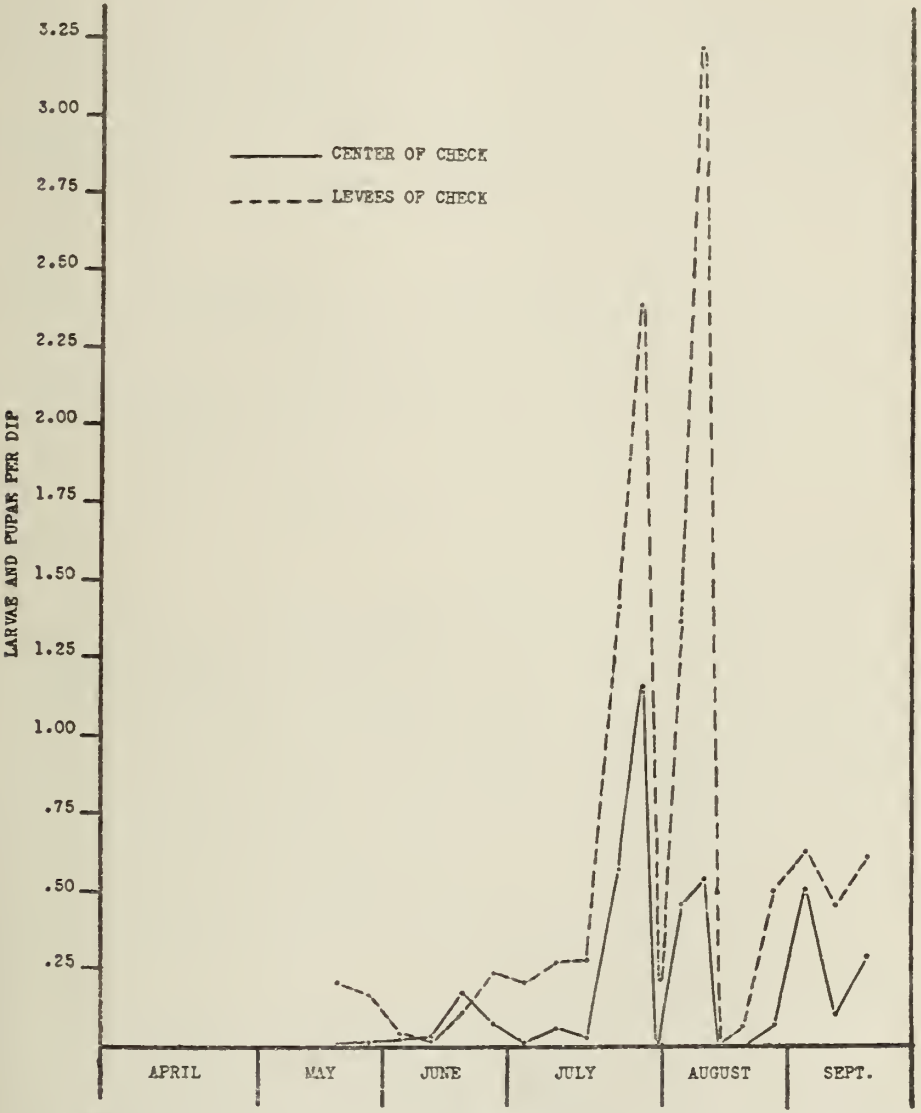

FIG. 9 Mean total number of mosquito larvae from center and levees of Frobose "check" (Spray check no. 2) April–September, 1947. (Weekly mean number of larvae and pupae per dip). "Check" sprayed July 29 and August 12, 1947.

FROBOSE "CHECK" (SPRAYED WITH DDD EMULSION)

This "check" contained a very poor stand of rice mixed with a heavy growth of cattails and barnyard grass. The mean total of larvae and pupae collected from the

center and levees from the entire "check" is shown in Figure 9. It was not as productive of larvae as the other "checks". However, it was productive of more species of mosquitoes. From larval and pupal collections made at frequent intervals from April through September, the following reared adults are listed in the order of their appearance: *C. tarsalis*, *A. freeborni*, *C. erythrothorax*, *Culex stigmatosoma* Dyar, and *Culiseta inornata* (Williston). All species were reared from both center and levee collections, except *C. inornata*, which was reared only from levee collections.

C. erythrothorax adults first appeared in collections made during the third week of July; *C. stigmatosoma* adults from collections made during the fourth week of July; and *C. inornata* from collections made during the first week of August.

This "check" was sprayed twice during the season (July 29 and August 12, 1947) with an aqueous emulsion of DDD.

It was observed that the *Culex* and *Anopheles* made their appearance in the various "checks" shortly after the rice had emerged and stood erect. The average height of the rice at this time was between 15 and 18 inches.

Excellent control of larvae was noticed following all of the spray applications during the season. This control was temporary, and was followed by rapid reinfestation, the result of egg-laying by mosquitoes flying in from surrounding territory.

SUMMARY AND CONCLUSIONS

Observations in rice fields in Stanislaus County, California, and adjoining areas, during the summer of 1947, revealed that

1. Rice fields are productive of large numbers of mosquitoes, which appear in three well-defined peaks. The *Aedes* mosquitoes, represented by *Aedes dorsalis* (Meigen), *Aedes nigromaculis* (Ludlow), and *Aedes vexans* (Meigen), appeared immediately after flooding, and then disappeared. In the early summer, *Culex tarsalis* Coquillett appeared, followed in the late summer by *Anopheles freeborni* Aitken.

2. *C. tarsalis* reached its peak during the last week of June and first week of July. *A. freeborni* reached its peak during August and September.

3. The centers of "checks" were found to be as productive of mosquito larvae as were the areas adjacent to the levees. Larvae of the above-mentioned species were found to be well distributed throughout the "check".

4. In view of the general distribution of mosquito larvae throughout the "checks", and because of the extensive areas involved, present control methods are virtually confined to the use of aerial sprays.

5. Aqueous emulsions of DDT and DDD, applied from an airplane at the rate of 0.3 pound per acre, gave excellent control for periods of one to two weeks.

6. The control of disease-bearing mosquitoes, such as those found in our studies, which breed in rice fields, is of prime public health importance, as they are known carriers of malaria and the encephalitides.

ACKNOWLEDGMENTS

The writer expresses his appreciation to Mr. Arve H. Dahl, California State Department of Public Health, for making this investigation possible; Miss Geraldine B. Edwards, for assisting in the planning of the field work and frequent consultation in

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RESUMEN Y CONCLUSIONES

Observaciones en arrozales de "Stanislaus County", California, y en áreas limítrofes durante el verano de 1947 revelaron que:

1. Los arrozales pueden producir grandes cantidades de mosquitos que aparecen en tres proporciones máximas bien definidas. Los mosquitos *Aedes* representados por *Aedes dorsalis*, (Meigen), *Aedes nigromaculis* (Ludlow) y *Aedes vexans* (Meigen) aparecieron immediatamente después de inundaciones y entonces desaparecieron. *Culex tarsalis* Coquillet apareció al empezar el verano y fué seguido más tarde por *Anófeles freeborni* Aitken.

2. *C. tarsalis* llegó a su proporción máxima durante la última semana de junio y primera de julio; *A. freeborni*, durante agosto y septiembre.

3. Los centros de "checks", áreas de cultivo cercadas por diques, produjeron tantas larvas como las áreas adyacentes a los diques. Se encontraron larvas pertenecientes a las especies mencionadas arriba bien distribuídas por todo el "check".

4. Debido a la distribución general de las larvas de mosquitos por todos los "checks" y debido a las áreas extensas consideradas, los presentes métodos de control están virtualmente limitados al uso de rociadas aéreas.

5. Emulsiones de DDT y DDD en agua aplicadas desde un aeroplano en una proporción de 0.3 de libra por acre lograron control excelente por períodos de una a dos semanas.

6. El control de mosquitos, portadores de enfermedades, como los encontrados en nuestros estudios, que se crían en arrozales, es de gran importancia en salud pública pues ellos son conocidos transmisores de malaria y encefalitis.

COMPARATIVE EVALUATION OF CERTAIN HIGH PRESSURE INSECTICIDAL AEROSOLS AGAINST *MUSCA DOMESTICA*

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Efforts have been made to improve the aerosol formulation G-382 (see table 1 for composition) now approved for use in controlling insects of medical importance in intercontinental aircraft. In developing possible alternative formulations, consideration has been given to cost, effectiveness, interrelationships of ingredients, and effects on aircraft finishes. To increase the accuracy of comparative evaluations, certain modifications have been introduced into existing procedures.

The house fly, *Musca domestica*, was used in the evaluation of insecticidal effectiveness of the aerosols since it was shown to be less susceptible to typical formulations than *Aedes aegypti* and *Anopheles quadrimaculatus*, the other available diptera. Also the house fly, being world-wide in distribution and readily enters planes, represents one of the principal species of insects encountered in intercontinental aircraft.

In developing formulations, consideration was given to newer products such as allethrin (the synthetic allyl homolog of cinerin I) as a possible replacement for natural pyrethrins, insecticide 264 (N-(2-ethylhexyl) bicyclo [2.2.1]-5-heptene-2,3-dicarboximide) and piperonyl butoxide as pyrethrum synergists, Lethane 384 (50 per cent solution of beta butoxy beta′ thiocyano diethyl ether in petroleum distillate) as an added insecticide, and Sovacide 544-G (Socony Vacuum Oil Co.) as a solvent.

TECHNIQUE

Comparative evaluations of test aerosols were made at 80°F and 70 per cent relative humidity in a modified Peet-Grady chamber having the walls, ceiling, and floor lined with Kraft Paper. Using a calibrated dispenser, 0.65 gram of a test aerosol was released into a Peet-Grady chamber, at a discharge rate equivalent to 3 grams per 1,000 cubic feet. Approximately 200 adult flies were introduced into the chamber immediately after the aerosol discharge and allowed to distribute themselves in the chamber. After a 15-minute exposure period, a 2-minute exhaust period, and a 3-minute waiting period, the insects were collected by means of a vacuum-cleaner type aspirator. Two collections of insects were made: (1) the flies knocked down by the insecticide within 20 minutes after the aerosol discharge, and (2) the flies remaining up after this time. All insects were supplied with food and held at 80°F and 70 per cent relative humidity for 24 hours. At this time comparisons were made of the female mortalities from the test formulation and the standard G-382 to obtain an index of effectiveness.

It was found necessary to expose a fresh paper on the walls and ceiling of the chamber at the beginning of each day and to renew the floor covering with each suc-

[1] From Technical Development Services, Savannah, Ga., with funds provided in part by the Foreign Quarantine Division.

cessive test to avoid any residual effects from the aerosol applications. Tests with free-flying house flies exposed for 15 minutes in the chamber showed 24-hour mortalities of 3 per cent of the males and none of the females with this procedure. However, if the floor paper was not changed with each application, the mortalities increased to 5 per cent of the males and 4 per cent of the females after one application, and 16 per cent of the males and 10 per cent of the females at the end of four applications. The aerosol dispenser was so designed that the complete dosage (0.65 gram) was delivered with a particle size range under 30 microns, 90 per cent of which were under 10 microns in diameter.

When flies were released in a Peet-Grady chamber, they immediately distributed themselves in the chamber but the pattern of this distribution varied from test to test. When the aerosol was discharged after the flies had distributed themselves within the chamber, the variation of distribution from test to test apparently influenced the results. On the basis of these observations, the flies were introduced near the floor of the chamber immediately after the discharge so that the majority of the flies flew through the aerosol. It has been demonstrated that insects flying through aerosols in this manner pick up more insecticide than resting or walking insects (David and Bracey, 1946). This initial dosage appears to have a stimulating effect on the insects and causes them to become restless rather than remaining motionless on the walls and ceiling. The importance of this change in technique was demonstrated by counts made of the relative number of droplets on carbon-coated slides at different levels within the chamber after a standard aerosol discharge. Counts made 15 minutes after an aerosol discharge showed a ratio of 1 to 200 between the number of droplets on slides at or near the ceiling level to those on slides on the floor. Slides mounted on the walls gave readings similar to those on the ceiling. These findings are in accordance with others on impingement of small droplets on solid objects (Johnstone *et al.*, 1949). This modification in technique greatly reduced variation between replications.

The 20-minute period between the discharge of aerosol and the collection of knockdown was selected because the knockdown and 24-hour mortality with the Standard G-382 were essentially equal at this interval.

Separate mortalities were recorded for each sex but only the female mortality was used in calculating the efficiency index of an aerosol. The males were not used because they were weaker than females and showed a wider range in daily susceptibility, especially at low dosage levels.

G-382 is recommended at a dosage of at least 5 grams per 1,000 cubic feet for control of insects of medical importance (Federal Security Agency, 1948). In Peet-Grady tests, this rate gave 24-hour mortalities of more than 80 per cent for female house flies. Since this value was too high for purposes of comparison, the dosage level of 3 grams of G-382 per 1,000 cubic feet, giving between 50 and 60 per cent female mortality, was adopted. The efficiency of a test aerosol was recorded as a standard G-382 index. This index is the ratio of the average per cent mortality obtained with the test aerosol to that obtained with G-382. Indices were calculated and results were based on determinations made the same day and with the same general group of flies.

A group of flies was subjected to all the mechanical operations of the testing technique as a check for noninsecticidal mortality.

RESULTS

In all comparisons, one aerosol formulation approved for use on aircraft, formula G-382, was adopted as the standard (table 1, Group A).

At the dosage rate of 3 grams per 1,000 cu. ft., G-382 produced 24-hour mortalities of 50 to 60 per cent of the adult female *M. domestica* exposed for 15 minutes.

TABLE 1

Proporional parts by weight of various aerosol ingredients and average indices

Each index was determined by a minimum of four paired replications with the standard formula G-382.

GROUP NO.	FORMULA NO.	INGREDIENTS, PERCENTAGE BY WEIGHT									AVERAGE 24-HR. FEMALE INDEX*
		Pyrethrum 20 per cent Extract	DDT	Lethane 384	Cyclohexanone	Sovacide 544-G	"264"	Piperonyl Butoxide	Lubricating Oil SAE #30	Freon 12	
A	G-382	5	3	—	5	—	—	—	2	85	1.0
	S-47	2	3	—	—	10	—	—	—	85	0.6
	S-46	2	3	—	—	9	1	—	—	85	0.9
	S-45	2	3	—	—	9	—	1	—	85	1.2
	S-52	2	3	—	—	8	—	2	—	85	1.2
B	S-45	2	3	—	—	9	—	1	—	85	1.2
	S-44	2	3	—	—	8	1	1	—	85	1.1
	S-50	2	3	—	—	7	2	1	—	85	0.9
	S-51	2	3	—	—	5	4	1	—	85	1.3
	S-60	2	3	—	—	3	6	1	—	85	1.3
	S-43	2	3	—	—	1	8	1	—	85	1.4
C	S-52	2	3	—	—	8	—	2	—	85	1.2
	S-57	2	3	—	—	4	4	2	—	85	1.5
	S-58	2	3	—	—	2	6	2	—	85	1.6
	S-59	2	3	—	—	—	8	2	—	85	1.3
D	S-67	2	—	4	—	9	—	—	—	85	0.04
	S-61	2	3	4	—	6	—	—	—	85	1.4
	S-63	2	3	4	—	5	—	1	—	85	1.7
	S-68	2	3	4	—	4	—	2	—	85	1.9

* Mortality (approximately 50 per cent) obtained with G-382. is taken as 1.0.

It has been reported (Fales *et al.*, 1946) that high pressure DDT aerosols with a 15 to 85 ratio of nonvolatile to volatile materials were the most effective. This ratio has been maintained in all the test formulations in the present paper. The results of the following studies were obtained from a minimum of four replications and from as many as 20 replications of the more promising formulations.

In the G-382 formulation the cyclohexanone acts as a solvent for DDT and the lubricating oil is included to secure proper particle size in the discharges. These components have certain disadvantages. Cyclohexanone causes crazing of stressed Plexiglas and has a solvent effect on other plastic finishes. The lubricating oil tends

to leave oily residues which are objectionable on certain finishes. In view of these factors several alkylated naphthalenes were tried as solvents for DDT. Of those tested, Sovacide 544-G[2] was found satisfactory since it did not craze Plexiglas when used in formulations and was a good DDT solvent. This solvent was used therefore in all the test formulations. With the Sovacide 544-G substituted for cyclohexanone and lubricating oil in G-382, the index of effectiveness was essentially 1.0.

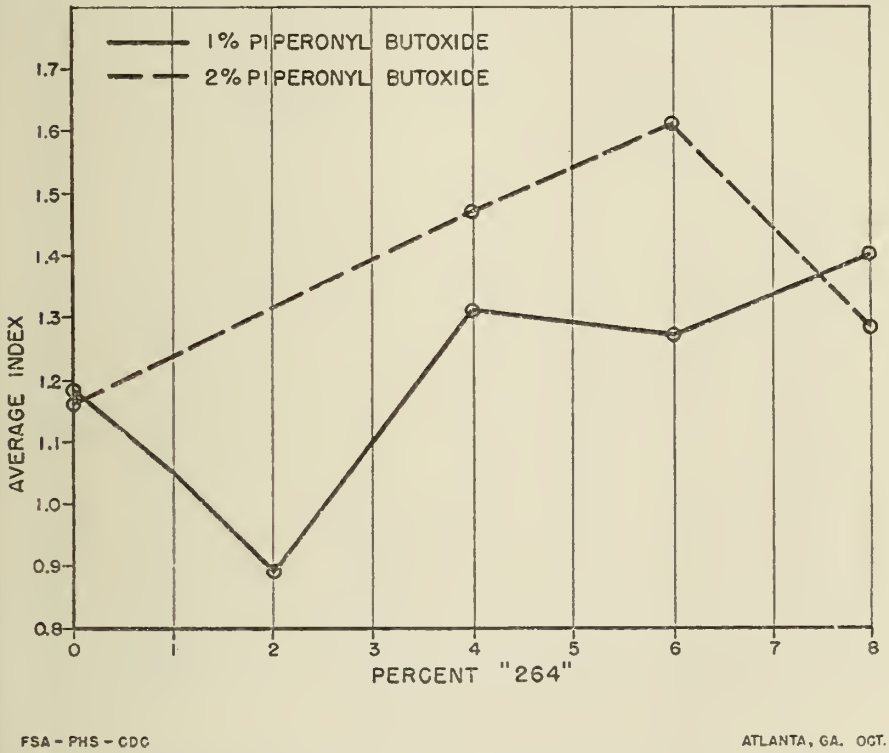

FIG. 1. Average index of effectiveness, against adult female house flies, of formulations containing two per cent pyrethrum extract, three per cent DDT, increasing percentages of "264", and either one or two per cent piperonyl butoxide. Mortality obtained with G-382 is taken as one.

Pyrethrum extract is expensive and currently somewhat limited in supply. If effectiveness could be obtained with less pyrethrum, it would be economically desirable. Therefore, a series of formulations with the pyrethrum content reduced to 2 per cent was evaluated. With no synergistic agents added, the initial formulation (S-47) gave a comparatively low index of 0.6. When small amounts of pyrethrum synergists were added, however, such as "264"[3] in formula S-46 or piperonyl butoxide[4] in formulae S-45 and S-52, the indices ranged from 0.9 to 1.2 (table 1, group A). The initial series showed that small amounts of pyrethrum synergists permitted the reduction of pyre-

[2] Product of Socony Vacuum Oil Co., New York, N. Y.
[3] Product of McLaughlin, Gormley King Co., Minneapolis, Minn.
[4] Product of U. S. Industrial Chemicals Corp., New York, N. Y.

thrum from 5 to 2 per cent without the reduction of effectiveness below that of G-382. Therefore, determinations of effectiveness of various combinations and concentrations of these synergists were made.

Insecticide 264 is not only a pyrethrum synergist but is also a good DDT solvent which does not craze Plexiglas. Therefore, large amounts can be used without the necessity of increasing the 15 to 85 ratio of the nonvolatile to volatile materials. In a second series (table 1, group B) piperonyl butoxide was maintained at one per cent and the proportion of "264" was increased progressively. The increased amounts of

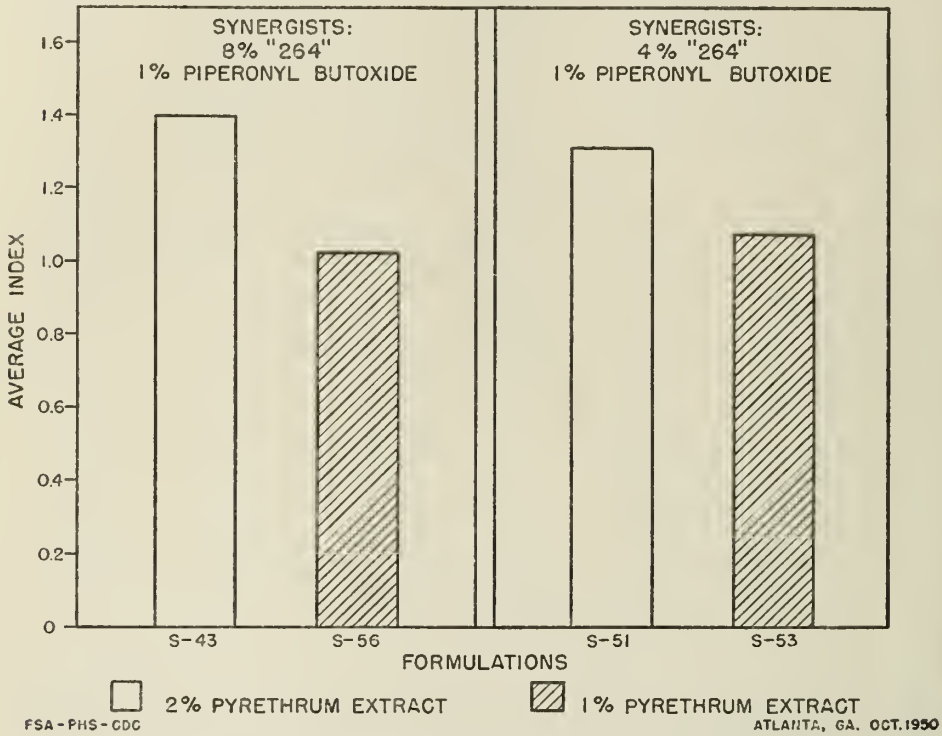

FIG. 2. Average index of effectiveness against adult female house flies of formulations containing one or two per cent pyrethrum extract plus combinations of synergists. Mortality obtained with G-382 is taken as one.

"264" replaced Sovacide 544-G as the DDT solvent. Formulations S-44 and S-50 containing one and two per cent "264" gave lower indices than did the formulation without "264" (S-45). Further additions gave increased indices with a peak of 1.4 a 8 per cent of "264" (S-43).

In a third series (table 1, group C) piperonyl butoxide was maintained at 2 per cent and "264" was increased progressively. The indices showed progressive increases to a peak of 1.6 at 6 per cent of "264" (S-58). Further addition of "264" (8 per cent concentration) decreased the index (S-59).

In general, with equal amounts of "264", formulations containing 2 per cent piperonyl butoxide were more effective than those with one per cent (figure 1). However,

piperonyl butoxide at a concentration of one per cent or higher produces irritation of the mucous membrane of the nose and throat. Consequently, these formulations are recommended for use in the disinsectization of international aircraft only in the absence of passengers.

Further reduction of the pyrethrum content to one per cent was tried with the more efficient synergist combinations, one per cent piperonyl butoxide with 4 per cent or 8 per cent "264" (S-53 and S-56, respectively). With this reduction of pyrethrum, in-

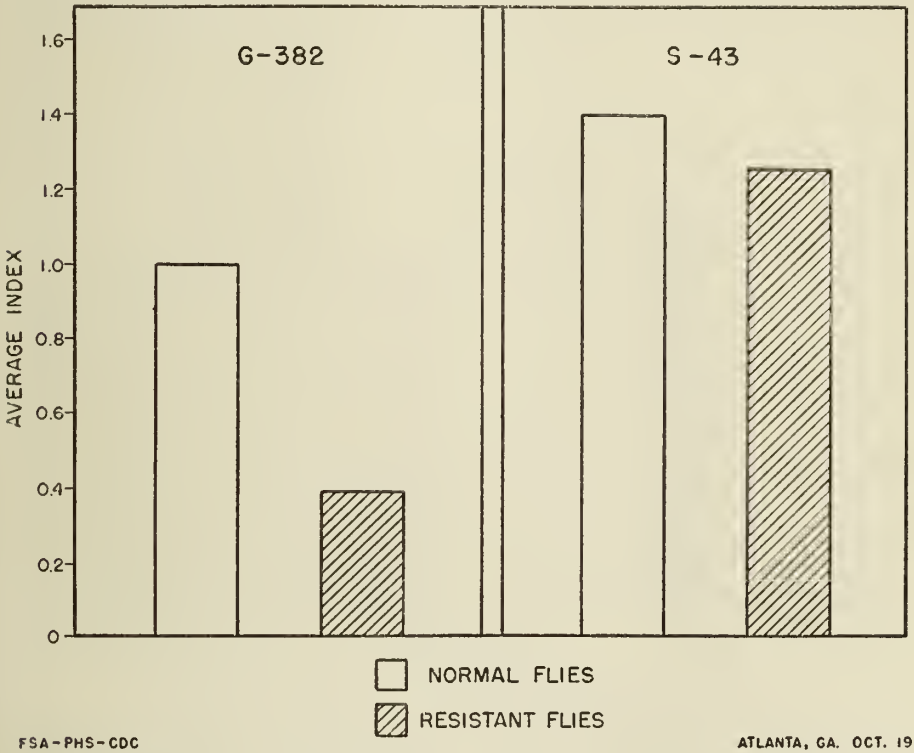

FIG. 3. Average index of effectiveness of G-382 and S-43 against normal and DDT-resistant strains of adult female house flies. Mortality obtained with G-582 against normal flies is taken as one.

dices went down to 1.1 and 1.0, respectively. A comparison between these formulations and their 2 per cent pyrethrum counterparts is shown in figure 2.

These results indicate that the proper combinations of "264" and piperonyl butoxide increase the efficiency of the formulations so that the concentration of pyrethrum can be dropped as low as one per cent and still maintain formulations equal to or better than G-382. The reduction of pyrethrum from 5 per cent to 2 per cent or one per cent make formulae of this type considerably cheaper than G-382. However, this type of formulation should be used in the absence of passengers as previously explained.

Since DDT-resistant house flies have been encountered in many parts of the world, two formulations, G-382 and experimental formula S-43 (table 1, group B) were

tested against a laboratory strain of DDT-resistant house flies (figure 3). The index of G-382 dropped from 1.0 against normal flies to 0.4 against resistant flies. The indices of S-43 were 1.4 and 1.3, respectively. The effects of the pyrethrum synergists were more evident with the DDT-resistant flies. Further tests with G-382 and formula S-43 were made using a field strain of house flies that had shown high resistance to DDT, chlordan, lindane, and dieldrin. The G-382 had an index of 0.3 while the S-43 had an index of 1.2.

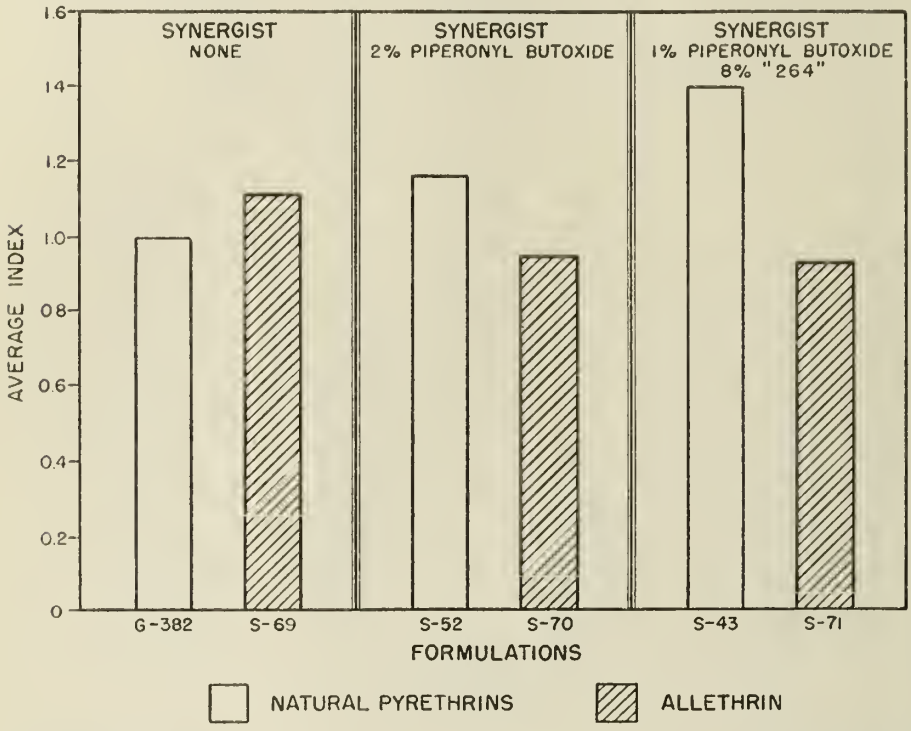

FIG. 4. Average index of effectiveness against adult female house flies of duplicate formulations containing natural pyrethrins or allethrin. Mortality obtained with G-382 is taken as one.

The substitution of allethrin, the synthetic allyl homolog of cinerin I, for natural pyrethrum in aerosol formulations was investigated. Substitution of either the purified homolog or the technical grade allethrin (U. S. Industrial chemicals) for the pyrethrum in formula G-382 gave an index of 1.1. The allethrin content was reduced to the equivalent of 2 per cent pyrethrum (0.4 per cent pyrethrins) and combined with piperonyl butoxide and "264". Comparisons between pyrethrum and allethrin in formulations without synergist, with 2 per cent piperonyl butoxide, and with one per cent piperonyl butoxide and 8 per cent "264" are shown in Figure 4. It appears that piperonyl butoxide and "264" do not have as great a synergistic effect on allethrin as they do on the pyrethrins.

With the licensing of Lethane 384 (Rohm and Haas Co.) for use in aerosol formu-

lations, it was tried in several combinations. At a concentration of 4 per cent in combination with 2 per cent pyrethrum extract (S-67), it gave an extremely low index of 0.04. With the addition of 3 per cent DDT to the above combination (S-61), the formulation proved highly effective with an index of 1.4. Further addition of one per cent piperonyl butoxide (S-63) gave an index of 1.7 and the addition of 2 per cent piperonyl butoxide (S-68) gave an index of 1.9 (table 1, group D) which closely approximates the maximum index possible when G-382 at 3 gm./1000 ft.^{3} is taken as 1.0. These preliminary results indicate that further investigations with this compound are warranted.

SUMMARY

1. A modified method has been utilized for the evaluation of various high pressure aerosols in modified Peet-Grady chambers against insectary reared *Musca domestica*.

2. Comparisons have been made between experimental formulations and G-382, an approved aircraft formulation, containing 5 per cent pyrethrum extract and 3 per cent DDT. A formula containing one per cent piperonyl butoxide, 8 per cent "264", 3 per cent DDT, and only 2 per cent pyrethrum was cheaper and more effective than the standard G-382, however it produced irritation of the mucous membrane of the nose and throat.

3. The combination of one per cent piperonyl butoxide and 8 per cent "264" as pyrethrum synergists has maintained the effectiveness of an experimental formula against house flies resistant to various halogenated hydrocarbons.

4. Allethrin has been substituted for pyrethrum in the standard G-382 formulation without loss in effectiveness. The synergistic action of piperonyl butoxide and "264", however, has not been as evident in formulations containing allethrin substituted for the natural pyrethrum.

5. The product Lethane 384 has shown considerable promise as an effective ingredient in aerosol formulations.

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RESUMEN

1. Se ha utilizado un método modificado para avaluar aerosoles de alta presión en cámaras "Peet-Grady" modificadas contra la *Musca domestica* de insectario.

2. Se han comparado formulaciones experimentales con el G-382, una formulación para aviones aprobada que contiene 5 por ciento de extracto de pelitre y 3 por ciento de DDT. Una formula compuesta de 1 por ciento de "piperonyl butoxide", 8 por ciento de "264", 3 por ciento de DDT y solamente 2 por ciento de pelitre resultó

más barata y más efectiva que el compuesto clásico G-382, pero produjo irritación de las membranas mucosas de la nariz y garganta.

3. La combinación de "piperonyl butoxide" al 1 por ciento y "264" al 8 por ciento como sinergistas de pelitre ha mantenido la efectividad de una formula experimental contra moscas de casa resistentes a varios hidrocarburos halogenados.

4. El pelitre ha sido substituído por "allethrin" en la formulación clásica G-382 sin reducir su efectividad. La acción sinergística de "piperonyl butoxide" y el "264", sin embargo, no ha sido tan evidente en formulaciones conteniendo "allethrin" que ha substituído el pelitre natural.

5. El producto "Lethane 384" ha prometido ser un ingrediente eficaz en formulaciones aerosoles.

SOME EPIDEMIOLOGICAL ASPECTS OF MALARIA CONTROL WITH REFERENCE TO DDT*

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In this paper an attempt has been made to describe and illustrate briefly the epidemiological significance throughout the world of the new residual insecticides typified by DDT.

DDT

Dichloro-diphenyl-trichloroethane was synthesized by Zeidler in Germany in 1874 but its insecticidal properties were discovered by Paul Müller in Basle in 1936–1937 (West and Campbell, 1950). The greatest importance of this compound, universally called DDT, lies in its prolonged residual effect when sprayed on surfaces upon which insects walk or rest, as first noted for houseflies by Wiesmann in Switzerland in 1942 (West and Campbell, 1950). The special influence of DDT on the epidemiology of malaria stems from the fact that once applied in a suitable formulation, it will for many months remain an effective destroyer of anopheline mosquitoes. Certain other toxicants, such as benzene hexachloride (BHC), chlordane, and dieldrin, have similar action but shorter residual periods.

Obviously, if a sufficient number of adults of a vector species acquire lethal doses of such toxicants during the period of sporozoite development, then the transmission of malaria in the community will be interrupted. Any measure which destroys infected vectors before they become *infective* will cut off transmission even though it may not measurably reduce the local density of adults or larvae, or even the numbers of vector adults collected inside or outside treated habitations. However, residual sprays tend to reduce the over-all age of a mosquito population, thus limiting ovipositing. This effect and the killing action greatly reduce the incidence of certain species which are closely house-haunting, for example *Anopheles darlingi* and *A. funestus*.

The first tests of DDT as a residual spray against adult mosquitoes appear to have been made in April, 1943, by Gahan and colleagues in Orlando, Florida (Gahan and Lindquist, 1945; Gahan *et al.*, 1945a and b). Field tests were started in August of that year near Tallahassee, Florida, and Stuttgart, Arkansas, and were continued in 1944. These studies dealt with the effect on anophelines rather than on malaria. Who first measured the effect of residual DDT on malaria has not been determined from the records. But numerous trials were made in 1944 by various units of the Allied Forces in Italy, India, Pacific Areas, South America, and elsewhere. The results left no doubt about the malaria control value of DDT. Hundreds of tons were used by the Allied Forces in 1945 and 1946, for airplane space spraying, for larviciding, and for the

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residual spraying of habitations. In 1945 DDT first became available for civilian use on a practical scale.

SOME MALARIOUS COUNTRIES BEFORE AND AFTER THE ADVENT OF DDT

North America

U. S. A.: As recently as 1912–1915 the U. S. Public Health Service (USPHS) in a survey of 12 southern states estimated that a million cases of malaria were occurring annually in a population group of 25 millions, with incidence rates as high as 40.9 per cent in the Mississippi delta. But by 1940 there had been a marked decline. Faust and De Bakey (1942) reported an average malaria mortality rate of 3.02 per 100,000 in 14 endemic states. This rate had been above 10 in 1921 and again in 1923 and 1936. Boyd (1941) wrote that "there is some suggestion that the lowest level of this recession, at least insofar as natural or spontaneous factors may have been operative, was reached as long as 10 years ago", i.e. about 1930.

The advent of DDT found malaria incidence in the United States, reduced to a very low level by the operation of such factors as greater use of screening, more frequent deviation of vector species to a larger and better stabled animal population, improvement in quality and use of antimalaria medication, increased spraying with household insecticides, shift of population to industrial centers, migration of negroes northward and expanded antimalaria and agricultural drainage. However, malaria was still endemic and between 1930 and 1940 it had shown its power to reinvade areas from which it had apparently altogether receded. Also, it is of interest that experiments indicated that there had been no weakening in the potentialities of the parasite-host-vector chain of transmission (Andrews, 1948).

DDT had its first practical application for malaria control in the United States in 1945 when the Communicable Diseases Center of the USPHS, co-operating with certain state health departments, began a joint program which by 1950 had applied some six million residual DDT sprayings (Andrews and Gilbertson, 1950). Since 1947 (Andrews and Gilbertson, 1948; Andrews, 1950) the objective has been to eradicate malaria as an endemic disease in the continental United States, a goal which appears to be near. In 1948–1949 during a 21 months' epidemiological study in the formerly highly malarious states of Alabama, Georgia, Mississippi, and South Carolina, the USPHS found only 59 cases of malaria which might have originated within the United States (Quinby, 1950). On further study only a few of these appear to have been actually autogenous. Thus malaria is obviously approaching an end point. For this reason, the USPHS requested the National Malaria Society in 1950 to formulate a definition of endemic malaria and obtained the following:

"Malaria may be assumed to be no longer endemic in any given area when no primary indigenous case has occurred there for three years, if reporting, including the name and address of the patient and diagnosing physician, and case finding are actively promoted and adequate investigations are carried out."

The term "primary indigenous" malaria refers to the first parasite positive evidence of an infection resulting from natural mosquito transmission in the given area.

The addition of DDT to the factors named above has upset what promised to become a prolonged condition of light endemicity with occasional areas or periods of moderately increased incidence. It now seems likely that within a few years malaria may be declared to be not endemic in the continental United States. Since Canada has been without malaria for some years, Mexico will then mark the northern limit of endemicity of the disease in North America.

A good deal of DDT is being used in Central America from Mexico to Panama (Downs *et al.*, 1950) and malaria, although still highly prevalent in some areas, is nevertheless being forced out of many communities. So, too, in the West Indies much is being done, including, for instance, a project for the complete eradication of malaria from Tobago.

South America

Venezuela: With two efficient and abundant vectors, *Anopheles darlingi* and *A. albimanus*, Venezuela was one of the most highly malarious countries in South America. In 1916, for example, malaria mortality rates exceeded 200 per 100,000 inhabitants in the Guayana zone, 250 in the coastal and mountainous areas, and 650 in the plains area. Between 1916 and 1945 there was a recession, partly spontaneous but in later years undoubtedly due in considerable degree to conspicuous improvement in economic conditions, to greatly augmented use of antimalaria drugs supplied by the government, and to increased application of anti-Anopheles measures. However, up to the advent of DDT, the country was still highly malarious. For instance, in an area of some 500,000 population in the states of Aragua, Carabobo, and Yaracuy, the average malaria death rate for the years 1941 to 1945 was 173 per 100,000 inhabitants (Gabaldón, 1949). Thanks to a progressive policy the disease was under forceful attack, yet there was no thought of obtaining more than malaria reduction by the maximum feasible control effort.

Under Gabaldón the Malaria Division of the Health Department began in 1945 to use residual DDT spraying "on as large a scale as possible, ignoring the initial test and control areas, usual when a new method is introduced" (Gabaldón, 1949). The extensive surveys made before 1945 permitted Gabaldón to develop a campaign which had as its objective the "total elimination" of malaria from Venezuela, a country situated, it must be remembered, in the tropics. So far as the records go, this appears to have been the world's first project for nation-wide eradication of malaria by residual DDT spraying.

Malaria mortality in Venezuela, in the area mentioned above dropped from the 173 average for the years 1941 to 1945, to 5 in 1948, 4 in 1949, and 2 in 1950. Spleen rates had fallen in some areas from 1941–1945 averages of between 72 and 98 per cent to between 2.6 and 15.5 per cent in 1948. Venezuela has in fact ceased to be a highly malarious country and seems likely, within a reasonable time, to be able to eliminate malaria as a public health problem.

Brazil: Here DDT residual spraying experiments were carried out in 1945 and

1946. Since 1947 a very large project has been developed by the National Malaria Service under Pinotti, whose staff numbers 8,000. With antimalaria funds equivalent to approximately 10.65 million dollars, more than two and a half million habitations were sprayed in 1950, using some 3,000 tons of DDT, and protecting some 17.76 million persons (Pinotti, 1951). The results have been excellent, particularly against *Anopheles darlingi*, which seems to have been eradicated from some regions. *A. albitarsis*, and *A. aquasalis* have also been notably affected. Even with *A. bellator* and *A. cruzii* the transmission index has been brought to zero in certain areas. For example, among 67,857 infants examined in 1949 in a number of areas formerly highly malarious, there were only 14 malaria positive smears. Malaria morbidity in all treated areas has sharply declined.

Elsewhere in South America DDT has also been extensively used. For example, in Argentina, thanks to DDT, Alvarado and his colleagues (1950) could conclude that "endemic malaria has therefore ceased to be a health and social problem in Argentina". Bolivia, Ecuador, Peru, and Colombia have extensive projects in hand (Pampana, 1950). In British Guiana, Giglioli (1948) with residual DDT has banished malaria from the populous coastal strip and, without larvicidal help, has apparently eliminated *A. darlingi*. Chile, never highly infested, had eradicated malaria before the DDT era. The last indigenous case was reported in April of 1945 (Pampana, 1950).

So the disease is in dramatic retreat in South America.

Europe

Italy: This country has a very long history of malaria and of attempts by Emperors, Popes, and the recent Dictator to control it. At the beginning of the century malaria was responsible for over 15,000 deaths each year. The following table (Missiroli and Moreschini, 1951) gives some interesting data:

YEAR	MALARIA PER 100,000 POPULATION	
	Morbidity rate	Mortality rate
1900	—	49.0
1902	544.2	30.3
1905	974.0	23.7
1910	585.4	10.5
1917	826.7	23.7
1925	723.8	9.2
1930	497.9	6.8
1935	382.2	4.0
1940	214.9	1.092
1942	362.9	2.377
1945	900.6	0.844
1947	462.9	0.204
1948	201.2	0.008
1949	42.2	zero
1950	7.5	zero

This table shows that until the DDT era malaria remained a serious threat to the country. The demonstrations of larviciding by Missiroli (1927, 1928, 1930) and

Hackett (1937) and the drainage works of Mussolini had brought morbidity rates down from 723.8 in 1925 to 214.9 in 1940 but when the war disrupted public services and the German Army sabotaged the drainage, the rate surged up again to a height of 900.6 in 1945.

Demonstrations by the Allied Control Commission Malaria Section in civilian areas in 1944–1945 and carried forward by UNRRA in 1946 indicated that malaria in Italy could be effectively controlled by DDT residual spraying. In January 1946 Missiroli, with great vision and real courage, announced a five-year plan aimed at no less than the complete eradication of malaria from Italy by the use of DDT residual spraying (Missiroli, 1950). Many thought the scheme fantastic but it was put into effect and has achieved the results predicted by Missiroli. Malaria in Italy, including Sicily, and Sardinia (where a separate *A. labranchiae* eradication scheme was carried out) is now an uncommon disease and for the two years 1949 and 1950 not a single malaria death was recorded, the first time in more than 2,000 years.

Greece: Malaria in Greece dates back to the Hippocratic period, and the disease had been highly endemic from that time until the advent of DDT. In 1905 it was estimated that in a total population of $2\frac{1}{2}$ million persons there were a million cases of malaria with some 6,000 deaths each year. There was no effective control until after the establishment of the Malaria Division of the Athens School of Hygiene in 1930. Under the guidance of Balfour, Livadas and others the principles of malaria control by mosquito reduction were demonstrated and both drainage and larvicidal measures were practiced. Malaria deaths per 100,000 inhabitants in Greece dropped from a high of 128.7 in 1923 to 44.2 in 1934 and 48.3 in 1937. But Greece continued to be highly malarious and during the war years it was intensely so (Livadas and Sphangos, 1941).

DDT has been used on a national scale since 1946. The program has included residual spraying and, in some areas, also airplane larviciding with DDT. The results have been such that Livadas in 1950 said malaria has "ceased to represent a grave public health problem" (Livadas *et al.*, 1950). Total malaria parasite indices were 1.76, 0.21, 0.21 and 0.15, and the infant parasite indices were 0.3, 0.0, 0.4, and 0.4 for the years 1946 to 1949 inclusive (ECA Mission, Greece, 1951).

So also in Corsica, Portugal and Yugoslavia effective control is being developed with DDT residual spraying (Pampana, 1950).

Cyprus, once highly malarious, is now completely free of the disease, a result achieved not primarily by residual spraying but by DDT larviciding which has almost eradicated anophelines from the island (Aziz, 1948).

DDT has been used successfully in the Netherlands, also in Germany, and Austria, and to a lesser extent in Spain and elsewhere. How much has been applied and with what effect behind the Iron Curtain has not been told. But obviously DDT has changed the malaria map of Europe.

Near East

A residual spraying program of considerable size is being carried out in Turkey very effectively. Israel also has malaria control well in hand. The World Health Organization (WHO) is operating malaria control demonstration teams in Lebanon and

Iran, and the use of DDT will doubtlessly be much greater in the near future. But DDT has not yet significantly modified the epidemiology of malaria in the Near East.

Asia

Except in Ceylon, DDT cannot yet be said to have had a nation-wide influence of the epidemiology of malaria in Asia. But the results in Ceylon, and in a portion of Bombay State, have been so strikingly good, and the preliminary trials of residual spraying elsewhere in India, and in Afganistan, Pakistan, Burma, Malaya, Thailand, Cambodia, Viet-nam, and Indochina have been so promising that one can safely predict a notable decrease in malaria in Asia within the next 10 to 20 years. Only in the Philippines, on the basis of trials not yet fully conclusive, residual DDT spraying has been declared to be of doubtful help in malaria control (Smith and Dy, 1949). One must also assume that in China and Indonesia the disease will remain relatively uncontrolled for the present.

Ceylon: In 1946 this country became the first in Asia to carry out a nation-wide malaria control scheme with residual DDT. Previously the disease had constituted the greatest public health problem. Occasionally, as in 1934, appalling malaria epidemics caused untold suffering and loss of life. Now a dramatic change is taking place. Rajendram (1950), Superintendent of the Antimalaria Campaigns, reports that the malaria death rate, which from 1940 to 1946 fluctuated between 854 and 1880 per 100,000, fell in 1947 to 663, in 1948 to 473, in 1949 to 329 and in 1950 to 215. At the same time the general mortality rate declined. In 1928 it was 24.8, in 1946, 20.3, in 1949, 12.6 and in 1950, 12.4. Infant mortality was 177 in 1928, 141 in 1946, 87 in 1949 and 79 in 1950.

The fact that malaria is coming under effective control has made possible a series of reconstruction projects in areas once highly infested. For instance, in the Batticaloa district the Gal Oya project, a miniature TVA, employs 50 American engineers who, with their families, reside where malaria used to be hyperendemic. They had not had a single case of malaria up to the end of 1949 (Pampana, 1950).

India: Sinton estimated malaria morbidity in India at 100 million cases annually, with one million deaths. Thanks to the Malaria Institute, under Covell, Singh and their predecessors, the epidemiology of malaria is well known and much training of personnel has been accomplished. Some outstanding malaria control projects were carried out in Delhi and Bombay cities and on certain tea gardens, and there were areas under attack in Mysore, Madras, and certain other states. But relative to the total problem, little progress had been made prior to 1946. In the 1930's pyrethrum spray-killing was under study in Delhi and South India. This method, while successful when applied against a determined house-haunting species like *A. culicifacies*, failed in areas where *A. fluviatilis* was the vector because many individuals of the latter species leave habitations after their nocturnal meals and thus are not affected by day-time indoor space sprays.

DDT was used experimentally in India by Allied Forces personnel in 1944 and by civilian workers in 1945. The first major routine civilian residual spraying was initiated in Bombay State in July 1946 and has been carried forward successfully by Viswanathan and Rao, under the Health Department. The project has been expanded

each year and now includes an area of some two million habitations and nine million inhabitants, and its annual cost is less than 10 cents per person protected (Viswanathan and Rao, 1949; Pampana, 1950; Viswanathan, 1950). Spleen rates have fallen from as much as 70 to 7 per cent, infant parasite rates have been reduced from 15 to less than 1 per cent. During 1949 Viswanathan estimated that some 500,000 cases of malaria were prevented.

Here, then, is effective control in India which points to the approach of significant changes in the epidemiology of malaria in Asia.

Australia and Pacific Islands

Malaria has had some importance in North Australia but had come under effective control prior to the DDT era. The disease is highly endemic in New Guinea, the Solomons and on into Indonesia. Not much use of DDT has been made since the Allied Forces left the islands and one may fairly say that the epidemiology of malaria has not yet been much affected by DDT.

Africa

The status of malaria in North and Central Africa has thus far been little changed by DDT. In Egypt, however, some areas, including the large oases, have had DDT residual spraying since 1945.

Extensive malaria control projects have been in progress for some years in the Union of South Africa under Annecke, and DDT has added much to the effectiveness of this work (Pampana, 1950). Malaria has not been eradicated but is coming under firm control. DDT has had successful trials in Southern Rhodesia and there is a large house-spraying program in Madagascar, involving a population of over a million (Pampana, 1950). On Mauritius, an Anopheles eradication project is being attempted, chiefly through residual spraying. Dowling (1950) reports that results have been excellent. The house spraying has not only brought malaria to a low incidence but appears also to have eradicated *A. funestus*, one of the two vectors. The density of *A. gambiae* does not yet seem to have been greatly changed, but in the presence of residual DDT the adults do not survive long enough to propagate malaria.

In some African experiments BHC has seemed more effective than DDT against *A. gambiae*. In a few studies neither toxicant has seemed to give results as effective as seen elsewhere. In general, DDT has not yet materially modified malaria epidemiology in Africa. However, many observers believe that residual spraying will undoubtedly prove to be useful in Central Africa, when given wider trials.

CONCLUSION

Undoubtedly, the advent of DDT has inaugurated a new and perhaps final stage in the long history of the intermittent fevers. In particular, the residual sprays have now demonstrated the economic feasibility of nation-wide malaria eradication, even where the disease has been most intensely prevalent. Already the epidemiology of malaria in some regions has been greatly modified and there is evidence that still wider areas will be cleared, first of severe malaria and finally of the disease itself. It is quite reasonable now to talk of its elimination, as a public health problem, from the world. Indeed, the WHO has announced this aim (Russell, 1950).

But there are two concluding thoughts which seem important. First, one recalls that malaria in the past has made spectacular reappearances and at times has widely overflowed its normal boundaries. Can it do so again? Secondly, increased population pressure follows malaria control. How can the sanitarian and the social scientist cooperate to take advantage of the absence of malaria in their efforts to advance toward a balanced human ecology, a goal which can never be attained in the presence of malaria?

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THE TOXICITY OF DDT TO *ANOPHELES CLAVIGER* (MEIGEN) IN SARDINIA AND ON THE ITALIAN MAINLAND[1]

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In the past several years an increasing number of papers have appeared reporting the development of acquired resistance by house flies and culicine mosquitoes to DDT and certain other insecticides, following the repeated exposure of these insects to these toxicants. It has become necessary to reconsider the role that these materials may play in the control, and particularly in the possible eradication, of various disease carrying insects.

Fay, Baker and Grainger (1949) have demonstrated in the laboratory a slight acquired resistance by adult *Anopheles quadrimaculatus* Say, following the exposure of one generation to residually treated surfaces, but they did not find any subsequent increase in resistance when they tested the offspring of the mosquitoes surviving exposure for the next three generations. When selective exposure was then omitted, the following generation reverted to the initial level of sensitivity to the toxicant. At this time, there are apparently no reports in the literature of the appearance of acquired resistance to DDT by the larvae of any anopheline species, although such resistance has been reported for several culicine species. A further search of the literature reveals that the basic minimum lethal dose (MLD) of various insecticides has been established almost entirely by experiments on a very few species of commonly colonized mosquitoes, such as *Anopheles quadrimaculatus* Say, *Culex quinquefasciatus* Say and *Aedes aegypti* (L.), although Bushland (1947) has reported results obtained with *Anopheles punctulatus* Dönitz, *Armigeres milnensis* Lee, and *Culex annulirostris* Skuse in New Guinea; and Yates (1950) has tested *Aedes nigromaculis* (Ludlow) and *Culex stigmatosoma* Dyar as well as mixed culture of *Aedes vexans* (Meig.) and *A. sticticus* (Meig.) in Oregon. The results obtained by Bushland (1947) indicate that the 48-hour MLD of DDT for *Anopheles punctulatus* in New Guinea was less than one part DDT to 40 million parts water, whereas the MLD of DDT for *A. quadrimaculatus* in the United States has been variously reported as being between 1 to 100 million and 1 to 200 million (see table 1).

Cognizance of the role that acquired resistance may play in the effectiveness of chlorinated insecticides in the field of medical entomology has made it increasingly important to determine the initial toxicity of these various chemicals to mosquito species of medical importance, as a means of establishing a base-line with which subsequently determined toxicities may be compared. The recent work of King (1950) would indicate that resistance on the part of adults may also be reflected in larvae;

[1] The studies on which this paper is based were conducted with the support and under the auspices of the International Health Division of The Rockefeller Foundation with the co-operation of the Italian government as part of the work of ERLAAS (Ente Regionale per la Lotta Anti-Anofelica in Sardegna).

at least he found this to be the case in *Aedes taeniorhynchus* (Wiedemann) and *Aedes sollicitans* (Walker). If the basic toxicity of various chemicals to mosquito larvae in different parts of the world is to be established for comparative purposes, it is desirable that a standard testing method be agreed upon. Particular attention should be given to standardizing the method of dispersing in water the toxicant under test, the volume of fluid used, the number of larvae per test, the temperature at which the test should be run, the duration of the exposure period, the number of replications which should be considered adequate, the question whether it is more desirable to express the results of the tests as MLD's (as has been most generally done) or as LD^{50}'s (which is the more usual pharmacological procedure) and whether the results are consistent enough to render the test valid for comparative purposes. Some variation in results obtained with different lots of larvae from a single laboratory colony has been reported.

In Sardinia, where an intensive program has been in progress over the past four years seeking the total eradication of the malaria vector species *Anopheles l. labranchiae* Falleroni, by all possible means, including both larviciding of water surfaces and residual spraying for adults, the question of a possible acquired resistance to DDT arose during the 1950 breeding season. Larvae of this species could not be obtained in adequate numbers for toxicity tests to establish the MLD, but adequate material was available for work on *Anopheles claviger* (Meigen). As the breeding places of this species as well as those of *A. l. labranchiae* has been extensively and repeatedly treated with DDT larvicides and as there was some doubt as to the effectiveness of the larvicidal treatments, it seemed desirable to determine the MLD of this species. The MLD of DDT to the larvae of this species had not previously been established. Therefore it was not possible to compare the results of the toxicity tests performed by me with similar results obtained before the intensive use of DDT in Sardinia. It was possible to establish, however, that DDT larvicides had not been used in the springs at Ninfa, in Latina Province on the Italian mainland, and to duplicate the toxicity tests performed on field-caught larvae of *A. claviger* from Ozieri, Sardinia, with larvae of the same species at Ninfa on the mainland.

METHODS

There is considerable variation in the methods various workers have used in making toxicity tests, and in how the results are reported. I have followed the methods most frequently used, within the limitations of the equipment available, so the results could be compared with those of other workers. DDT was dissolved in acetone in such proportions that the acetone-DDT solutions could be mixed with 250 cc. of water, so that not more than one cc. of the acetone solution was used to obtain the desired strongest concentration of DDT in water. Other workers have indicated that as much as one per cent of acetone in water was not toxic to mosquito larvae. The tests reported in this paper were conducted in 400 cc. beakers; 250 cc. of water was poured into each. About 25 cc. of water was then poured from each of these into adjacent 50 cc. beakers and 20 early fourth-instar larvae were introduced into the small beakers. The appropriate amount of DDT-acetone solution to obtain the desired concentration of DDT was then added to the remaining water in the 400 cc. beakers, and the mix-

ture stirred with a glass rod. The larvae and water contained in the small beakers were then poured into the large beakers. This procedure was used to avoid the shock of introducing the larvae directly into the DDT-water suspension. In all test runs two controls were used; one with the larvae in water alone, and one with the larvae in water to which had been added the maximum amount of acetone used to obtain the highest concentration of DDT-acetone in that test run. In Sardinia the tests were run in tap water from the city supply at Cagliari, while at Ninfa clear spring water in

TABLE 1

Average 48-hour mortality of 4th instar anopheline larvae in DDT-acetone water suspensions

(Results are given in per cent.)

Species	*Anopheles claviger*		*Anopheles quadrimaculatus*									*Anopheles punctulatus*
Location	Ozieri, Sardinia, Italy	Ninfa, Latina, Italy	Florida, U. S. A.									New Guinea
No. of replications	5	4	—	3	3	3	3	3	4	5	3	1 or 2
Parts DDT per million parts water.												
1 to 2.5	100											
1 to 10	98	95										
1 to 25	85	73										
1 to 40												90
1 to 50	69	55										90
1 to 75												80
1 to 100	45	35				98		100	100	100		45
1 to 200	10	20	98	97	84	71	100	100	100	94	98	30
1 to 300				93								
1 to 400			85	82	53	24	100	68	61	55	76	10
1 to 600											41	
1 to 800					16		19					
Authority for test			1	2	3	3	3	4	4	5	6	7

Authorities for the data given under *A. quadrimaculatus* and *A. punctulatus* are as follows: 1 = Deonier, Jones and Incho (1946); 2 = Deonier and Jones (1946); 3 = Incho and Deonier (1950); 4 = Deonier, Raun, Peek, Davis and Nottingham (1949); 5 = Deonier, Maple, Jones, Hinchey and Edie (1945); 6 = Incho and Deonier (1947); 7 = Bushland (1947). Percentages are given to the nearest whole number.

which the larvae were living was used. The larval mortality was read at 48 hours. Any larvae which had pupated during the 48-hour test period were omitted from the calculations of the results, as pupae are less sensitive to DDT suspensions than larvae. All tests were run at room temperature which maintained the liquids used in the tests at an average temperature of 22° to 23°C. As the chemical used was a sample of technical grade DDT, recrystalized DDT not being available, it was desirable to check this material for its toxicity. A portion of the sample of DDT used in these tests was checked by Dr. W. McDuffie in comparison tests with recrystallized DDT against

A. quadrimaculatus at the Orlando, Florida, laboratory of the U. S. Department of Agriculture, where it produced the same mortality as the standard material in use there.

RESULTS

The results of the tests are given in table 1 along with comparable data from the literature. Two interesting results are to be noted:

1. The 48-hour MLD of DDT for *A. claviger* is about 1 part DDT to 10 million parts water. The work of other investigators, whose results are summarized in Table I, indicates that the 48-hour MLD of DDT for *A. quadrimaculatus* is about one part DDT in 200 million parts water. It would appear, therefore, that *A. claviger* is only about one-twentieth as sensitive to DDT as is *A. quadrimaculatus*.

2. Larvae of *Anopheles claviger* from Ozieri in Sardinia, where DDT larvicides have been intensively and repeatedly used, and larvae of this species from Ninfa on the Italian mainland, where they have not been used, are about equally sensitive to DDT. There is no evidence of an acquired resistance in the Sardinian larvae of this species.

As a check on these results, toxicity tests were run on larvae of *Culex molestus* Forskal (= *Culex pipiens* var. *autogenicus* Roubaud) obtained near Cagliari, in the same beakers with *A. claviger*. While *A. quadrimaculatus* has been reported to be more sensitive to DDT than *Culex quinquefasciatus* (= *Culex fatigans* Wiedemann) by all authors working with these two species (Incho and Deonier, 1947; Deonier et al., 1949), the writer found the reverse to be true in the comparison of *A. claviger* with *C. molestus;* the MLD for *C. molestus* approximated that reported for *C. quinquefasciatus*.

SUMMARY

The 48-hour MLD of DDT for *Anopheles claviger* in Sardinia and on the Italian mainland was found to be one part DDT in 10 million parts of water, about one twentieth that reported for *A. quadrimaculatus* in the United States. Despite this relative lack of sensitivity of *A. claviger* to DDT, no evidence was found of an acquired resistance in Sardinia, where DDT has been intensively and repeatedly used, since the same MLD was found for larvae from an area on the Italian mainland, where DDT larvicide had not been used.

It would be desirable to establish the MLD (or the LD^{50}) of DDT and the other recently developed synthetic insecticides against the larvae of the important malaria vectors of the world, to fix a base-line from which any subsequently developed resistance might be measured. Methods of conducting and reporting toxicity tests against mosquito larvae should be standardized, for ready comparison of results from different parts of the world.

SOMMARIO

La MLD di DDT di 48 ore contra l'*Anopheles claviger* in Sardegna e nell'Italia continentale si è vista composta di 1 parte DDT in 10 milioni di parti d'acqua, per cui la sensibilità del *claviger* è circa una ventesima parte di quella riportata per *A. quadrimaculatus* negli Stati Uniti. Malgrado questa relativa mancanza di sensibilità dell'*A. claviger* al DDT non si è trovata nessuna prova di una resistenza acquistata in Sardegna, dove il DDT è stato usato intensivamente e repetutamente, dacche la

stessa MLD fu trovata per le larve di una zona nell'Italia continentale, dove il larvicida DDT non era stato usato.

Sarebbe desiderabile di stabilire la MLD ("dose minima mortale"), o la LD^{50} ("dose mortale, 50 percento") di DDT, e degli altri insetticidi sintetici recentemente prodotti contra gli importanti vettori di malaria, allo scopo di fissare una base dalle quale misurare qualsiasi resistenza che si sviluppasse nel futuro. I metodi per condurre e riportare le prove di tossicità contra le larve della zanzara dovrebbero essere standardizzati per poter prontamente comparare i risultati ottenuti nelle diverse parti del mondo.

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RESUMEN

La dosis letal mínima cuarenta y ocho horas (48-hour MLD) de DDT para *Anopheles claviger* en Sardinia y en el Continente Italiano resultó ser una parte de DDT por 10 millones de partes de agua, aproximadamente una vigésima parte de lo reportado en Estados Unidos para *Anopheles quadrimaculatus*. A pesar de esta relativa falta de sensibilidad de *A. claviger* al DDT no se notó ninguna evidencia de

resistencia adquirida en Sardinia, donde DDT se había usado extensamente y repetidamente pues se observó la misma dosis letal mínima contra larvas procedentes de una área del Continente Italiano donde el larvicida DDT no se había usado previamente.

Resultaría conveniente establecer la dosis letal mínima (o la dosis letal 50) de DDT y de otros insecticidas sintéticos descubiertos recientemente contra las larvas de los importantes vectores de malaria en el mundo para fijar una línea de base que serviría para medir resistencias desarrolladas subsiguientemente. Los métodos usados en la ejecución y reporte de pruebas de toxicidad contra las larvas de mosquitos deben ser normalizadas para lograr comparaciones ligeras de los resultados obtenidos en diferentes partes del mundo.

Herman Otto Proske

1890–1950

Herman Otto Proske, a fellow member of the National Malaria Society and Biochemist of the Health and Safety Division of the Tennessee Valley Authority, succumbed on December 23, 1950, to an acute heart attack while visiting his daughter in New Orleans, Louisiana.

Mr. Proske was born June 7, 1890, in Upper Silesia, Germany, and came to the United States in 1914. He received his training at the Johns Hopkins School of Medical Zoology, Hygiene, and Public Health. Later, he served as Director of Clinical Laboratories at the University of Maryland, at Franklin Square Hospital in Baltimore; at the U. S. Public Health Service, Venereal Disease Clinic, Hot Springs, Arkansas, and at the U. S. Bureau of Mines Cooperative Clinic at Picker, Oklahoma. In addition, he participated in Bacteriological and Epidemiological Investigations of the City Health Edpartment of Hot Springs, Arkansas, and in Industrial Hygiene Investigations of the U. S. Public Health Service at Washington, D. C. In 1936 he joined the staff of the Tennessee Valley Authority and served as a Bacteriologist and Biochemist. Mr. Proske was well known for his contributions to the welfare of his fellowman and developed a biochemical diagnostic test for malaria, and further contributed to a better understanding of blood chemistry associated with malaria infections.

When Mr. Proske's life came to an end, the Society lost a member who made significant contributions in his field and his associates lost a loyal and beloved friend.—*E. L. Bishop*

Eugene Lindsay Bishop

1886–1951

Dr. Eugene Lindsay Bishop, Director of Health and Safety of the Tennessee Valley Authority, died 27 February 1951, at Vanderbilt Hospital, Nashville, Tennessee.

He was born in the same city on April 3, 1886. His medical education was acquired at the University of Tennessee and Vanderbilt University Medical School where his M. D. was conferred in 1914. After a brief period of private practice, Dr. Bishop joined the staff of the Tennessee State Health Department in 1916 as Field Director of Rural Sanitation. Thus he commenced his life-time career in preventive medicine and public health.

He was promoted to the State Directorship of the Division of Rural Sanitation in 1918 and in that capacity established in 1919 the first rural full-time local health department in Tennessee. He promptly distinguished himself by his intensive and unremitting efforts to increase the quantity and improve the quality of health services available to the rural population of Tennessee. As a Fellow of the International Health Division of the Rockefeller Foundation, he spent a year in graduate study at the Johns Hopkins School of Hygiene and Public Health where he received in 1923 what is now known as the Master of Public Health degree. He then returned to his original post in Tennessee, serving also as Assistant Commissioner of Health.

In December, 1924, he was appointed State Commissioner of Health, a position which he held with distinction for ten years. During his tenure, he increased the percentage of full-time health protection for the rural population of Tennessee from 18 per cent to 57 per cent, an increase of more than two-fold.

Dr. Bishop was selected to head the Division of Health and Safety of the Tennessee Valley Authority in 1935. He accepted the position because it provided another opportunity for pioneering in a new field of service. Among the many important health problems with which he was confronted was the increase in malaria potential, the result of ecologic changes due to the conversion of flowing streams into quiet lakes.

The malaria problem in the Tennessee River Valley was a formidable one. It involved the surveillance and management of more than 10,000 miles of shoreline, much of which presented high potentials for anopheline production in traditionally malarious areas. Parasite rates varied up to 28 per cent in different sectors. Dr. Bishop attacked the problem by obtaining the advice of nationally-known malaria consultants from federal, state, foundation, and university agencies. They specified a program of combined research and operation in the field and in the laboratory. This was put into effect and carefully followed by Dr. Bishop, with the result that the effectiveness of malaria prevention increased as the unit costs of this protection decreased. Parasitism became virtually undiscoverable. The program attracted wide attention and TVA became one of the "musts" for foreign visitors to the U.S.A. interested in seeing up-to-date, efficient malaria control practice.

Dr. Bishop was a well-known speaker and contributed generously to the literature on malaria prevention and public health practice. At the time of his death, he was a

Fellow of the American Association for the Advancement of Science, the American Medical Association, and the American Public Health Association. He received his Diplomate from the American Board of Preventive Medicine and Public Health in 1949. At the 1950 meetings of the American Public Health Association he was given a Lasker Award "in recognition of his outstanding contributions in the field of public health administration." Dr. Bishop was a member of the American Social Hygiene Association, the Southern Medical Association, and the National Malaria Society. He became affiliated with the National Malaria Committee in 1925. He was named Chairman in 1932 and served on many subcommittees over the years. At the last meeting of this Society, he was elected Vice-President for 1951.

Dr. Bishop was distinguished not only for his organizing and administrative ability but for the charm of his personality. He was a man of quiet dignity and penetrating wisdom. He was well-known for his logical summations forcefully expressed in succinct but elegant language. The National Malaria Society mourns the passing of an able counsellor and friend.—*Justin M. Andrews*

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